Movement behaviour of traditionally managed cattle in the Eastern Province of Zambia: investigations using two-dimensional motion sensors

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DECLARATION

I, Caesar Himbayi Lubaba, do declare that the research described in this thesis is my own work and that it has not been submitted for any other degree or professional qualification.

Signed.....

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LIST OF ABBREVIATIONS

AAT	African Animal Trypanosomiasis
ANOVA	Analysis of Variance
bp	Base pair
CSF	Cerebrospinal fluid
CI	Confidence Interval
CNS	Central Nervous System
CTVM	Centre for Tropical Veterinary Medicine
CVRI	Central Veterinary Research Institute
dl	Decilitre
DNA	Deoxyribonucleic Acid
DVO	District Veterinary Officer
DVLD	Department of Veterinary and Livestock Development
ECF	East Coast Fever
EDTA	ethylenediaminetetraacetic acid
ELISA	Enzyme-Linked Immunobsorbent Assay
EU	European Union
FAO	Food and Agriculture Organisation
FTA	Flinders Technology Australia
g/dl	Grams per decilitre
GDP	Gross Domestic Product
GIS	Geographical Information System
GPS	Global Positioning System
HAT	Human African Trypanosomiasis
Hb	Haemoglobin
IFAT	Indirect Fluorescent antibody Test

ILRAD	International Laboratory for Research on Animal Diseases
ILRI	International Livestock Research Institute
Kg	Kilogram
MgCI2	Magnesium Chloride
ml	Millilitre
mm	Millimetre
NALEIC	National Livestock Epidemiology and Information Centre
NGO	Non-Governmental Organisation
OIE	World Organisation for Animal Health
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
RBC	Red Blood Cell
RTTCP	Regional Tsetse and Trypanosomiasis Control Programme
SIT	Sterile Insect Technique
Spp	Species
StDev	Standard Deviation
Taq	Thermus aquaticus
UK	United Kingdom
US$	United States Dollar
USA	United States of America
UV	Ultraviolet
VA	Veterinary Assistant
VO	Veterinary Officer
WBC	White Blood Cell
WHO	World Health Organisation
ZMK	Zambian Kwacha
μL	MicroLitre

ABSTRACT

Two-dimensional (2-D) motion sensors are activity motion sensors that use electronic accelerometers to record the lying, standing and walking behaviour of animals. They were used in this study with the aim of monitoring and quantifying the movement behaviour of traditionally managed cattle in the context of improving animal health and production in rural sub-Saharan Africa. Improvements in animal health and production could be made if data can be automatically collected on large numbers of animals and over prolonged periods of time. This data can then be used by stakeholders in making management and disease control decisions. This study was designed to assess whether the 2-D motion sensors were suitable for use on traditionally managed cattle in Kasero and Makale, two veterinary camps in Petauke District, Eastern Province of Zambia. It further aimed to provide a baseline for future research on traditional cattle movement behaviour. The study was carried out in a region where trypanosomiasis and tick-borne diseases are endemic and low haemoglobin values are often associated with these and other parasitic infections. An assessment was made on the effect on cattle movement behaviour of a treatment directed against tsetse-transmitted (*trypanosoma congolense* (Savannah type), *trypanosoma vivax* and *trypanosoma brucei*), tick-transmitted (*theileria parva*, a*naplasma spp*. and *babesia spp*.) and pasture-transmitted pathogens of African cattle. A structured questionnaire on livestock ownership and management practices showed that cattle owners considered trypanosomiasis and theileriosis the main constraints to improved cattle health and production in their traditional crop-livestock mixed farming system. A baseline study was conducted in which haemoglobin values were measured in 432 cattle in the two areas. In each area,

ten pairs of co-grazing cattle were selected on the basis of one high and one low haemoglobin value in each pair. The co-grazing pairs were age and sex matched. Each animal had a motion sensor placed on its hind leg, to continuously measure and record its activity for two weeks. There were significant differences in haemoglobin levels between the two camps with Makale having lower levels than Kasero. Baseline data indicated that a larger proportion of sampled animals in Makale had trypanosomiasis while those in Kasero had theileriosis. Molecular parasitological results showed that the proportion of animals sampled in Makale that had trypanosomiasis was greater (21.4% [95%CI: 16.4 – 27.1]) than that in Kasero (1.4% [95%CI: 0.5 – 4.1]). However, Kasero had a greater proportion of animals positive for theileriosis (25.6% [95%CI: 20.2 – 31.9]) than Makale (2.4% [95%CI: 1.0 – 5.2]). A total of 204 cattle were screened for a three week treatment study in Makale. From this number, 40 animals with low circulating haemoglobin levels (<8g/dl) were paired and investigated for differences in movement behaviour patterns between treated and non- treated cattle. Analysing the sensor data using principal components analysis (PCA) revealed that the treated animals (which had higher mean haemoglobin values at the end of the study) were clustered more closely on the score plots than the control animals (which had lower mean haemoglobin values). The numbers of steps taken by high haemoglobin cattle in both studies were significantly higher than the low haemoglobin cattle. This, coupled with the PCA results suggests an association between cattle haemoglobin levels and their movement patterns.

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Abstract

CHAPTER ONE

Movement Behaviour, Anaemia and Endemic Diseases of Cattle in Sub-Saharan Africa

1. Introduction and Literature Review

1.1 Role of cattle in sub-Saharan African society

Cattle are the mainstay of the agricultural and livestock industries in sub-Saharan Africa (Maule, 1990). They provide much of the draft power for farm work, haulage and water lifting and are adapted to surviving in harsh conditions. The bulk of the meat and milk (and dairy products) produced in many regions come from cattle (Maule, 1990; Trail et al., 1985).

There are over 300 breeds of cattle in the tropics numbering over 740 million (Maule, 1990). European breeds of cattle have been carefully selected for and are easily recognisable by, such characters as coat colour, length or shape of horns and other characteristics such as dairy or beef conformation. In the tropics, many local cattle populations vary widely in their general appearance (especially coat colour) although they are broadly of the same general type and origin (Maule, 1990).

Cattle play an important role in the lives of rural people in sub-Saharan Africa. There are many reasons why people especially in rural areas of Africa keep cattle. Cattle provide many benefits such as draft power for transportation and cultivation; manure for fuel and improving soil fertility; meat, milk and eggs for consumption or sale (Anteneh Addis 1987 ; Perry et al., 2002). Cattle are also the major source of food security and income for resource poor farmers in this region. Hides, blood, horns and dung are all useful by-products (Maule, 1990). Furthermore, livestock can enhance human capital and play a critical role in reducing malnutrition (IFAD, 2004).

In Zambia, agriculture contributes about 18% to the Gross Domestic Product (GDP) and some 75 percent of the population is engaged in agriculture (Central Statistical Office, 2000). Out of all the households raising livestock in the country, cattle raising households make up 35.2% (Central Statistical Office, 2000). There is a wide range of reasons for which households keep cattle. The reasons vary across households, and reflect the individual household's needs either directly such as provision of meat and milk or indirectly such as provision of income (Nkhori, 2004). Cattle are also kept for cultural reasons such as in the payment of lobola (dowry) during weddings, slaughter during ceremonies and funerals and for prestige (Nkhori, 2004). Common to the crop-livestock production systems is the interdependence of livestock and other forms of agriculture, with manure and draft inputs to crops being more important to the system as a whole than is milk production (Minjauw and Mcleod, 2003). For many poor households, keeping livestock is the primary form of savings. As an investment, few other resources can match livestock as a means of capital growth (IFAD, 2004).

1.1.1 Cattle indigenous to sub-Saharan Africa

Indigenous breeds of cattle generally possess a higher level of overall adaptation than exotic breeds to the stressful climatic, nutritive and disease-parasite environments that characterize much of the tropics (Maule, 1990; Trail et al., 1985). Cattle are classified into two main types as either *Bos indicus* or *Bos taurus* (Hill et al., 2001). Although both cattle types show marked phenotypic variation and have been recognized as separate species, the composition of crossbreeds is difficult to ascertain unless detailed records on their siring are available (Hill et al., 2001). Classification of the main types of cattle includes the humped cattle (Zebu and Sanga), humpless and crosses between them. The most common cattle are the humped, either Zebu (*Bos indicus*) or Sanga (any combination of zebu and

taurine) (Hill et al., 2001; Maule, 1990). Zebu and taurine breeds are differentiated primarily by the presence or absence of a hump (Loftus et al., 1994). The Zebu cattle have large thoracic humps while the Sanga have small cervico-thoracic humps (Gregory et al., 1985a, b).

The Zebu cattle are found in most parts of the tropics while the Sanga are only found in Africa. Zebu cattle in the Indian subcontinent number about 200 million and Zebu and Sanga cattle in Africa about 160 million (Maule, 1990). In Africa, Zebu cattle are important in East and North East Africa where they outnumber all other types, and in West Africa, although their distribution is restricted by the tsetse fly (Maule, 1990).

The origin of domesticated cattle is controversial. However, the most widely held view is that both types of cattle (*Bos indicus* and *Bos taurus*) derive from a single domestication event 8,000 to 10,000 years ago (Loftus et al., 1994). Longhorn, taurine animals in Africa are believed to represent the descendants of the herds maintained by the earliest husbanders on the continent, having emerged from the domestication centres of the Middle East some 7,000 years ago (Hill et al., 2001). Shorthorn taurine cattle on the other hand are thought to have appeared more recently in Africa (Hill et al., 2001). Zebu and zebu-derived cattle came to predominate in Eastern Africa after their introduction to the continent beginning about 3,500 years ago (Hill et al., 2001). Africa presently represents a mosaic of various cattle types ranging from the Asiatic zebu cattle through crossbred Sanga cattle to the taurine populations of West and Central Africa which have maintained their genetic integrity due to their tolerance to trypanosomiasis and other disease challenges (Hill et al., 2001).

1.2 Movement of cattle

The movement of cattle from one area to another depends on many factors. Cattle may be moved from one area to another by livestock keepers for reasons such as transhumance. Transhumance is the seasonal moving of livestock to regions of different climate and is an integral part of livestock production in many parts of the world (Eckert and Hertzberg, 1994). Movement of animals is in most cases in search of pasture and water. The Tonga people of southern Zambia are agro-pastoralists who practice transhumance by splitting their herd, leaving the "milk" herd permanently at their villages, and sending the rest of their animals on transhumance (Niamir, 1990). Droving is another form of movement of cattle over long distances (Blackman, 1975). In the early 1800's, Scottish cattle farmers practised long-distance droving of their animals on the hoof to markets in Yorkshire and London in England (Blackman, 1975).

The use of animal traction has been identified as an appropriate, affordable and sustainable technology to alleviate the drudgery in farm transport and reduce the associated crop post-harvest losses (Ayo-Odongo et al., 2000). Cattle movement by draft power is for agricultural or transport purposes. In countries that practice a mainly crop-livestock system such as in Eastern and Southern Africa, draft power is generally valued more highly than milk production (Minjauw and Mcleod, 2003). In the Eastern Province of Zambia, cattle draft power is mainly utilized in the form of cultivation of fields (Robinson et al., 2002). Management decisions by the livestock keeper will affect the movement of cattle in a given area. These management decisions may be influenced by factors such as a drought when the farmer has to herd his cattle over long distances in search of water and grazing areas. Outbreaks of a cattle disease in an area may influence

the farmer to move his cattle to another area/village in the hope that his animals will not be affected (personal observation).

1.2.1 Cattle found in the Eastern Province of Zambia

Figure 1. 1 Angoni cattle in Petauke District, Eastern Province, Zambia

The Angoni cattle (Figure 1. 1) are short horned Zebus found in the Eastern Province of Zambia and adjoining areas of Malawi between the Luangwa River in the west and Lake Malawi in the east spreading southwards reaching north-western Mozambique (Igboeli and Rakha, 1971; Maule, 1990; Mwenya, 2007). These cattle are today known as Angoni in Zambia, Malawi Zebu in Malawi, and Angone in Mozambique. The Angoni is larger than the other types (Maule, 1990; Mwenya, 2007). The colour of the Zambian Angoni varies considerably and may be red, brown, black or black and white, or brindle. Horns are short and thick

and lateral rather than upright (as in the Malawi Zebu); hump and dewlap are well developed (Maule, 1990). The Angoni cattle are triple purpose for meat, work and milk (Aregheore, 2007). The cattle are kept by the Ngoni people and other tribes of the Eastern Province of Zambia. The Zambian Angoni are medium sized cattle with cows weighing between 300 to 350 kilograms while bulls weigh 550 to 650 kilograms (Maule, 1990).

They are well adapted to a wide range of conditions and are known for their ability to produce a calf every year under low input traditional husbandry systems (Aregheore, 2007; Maule, 1990). Angoni cattle are considered the best indigenous beef cattle in Zambia, Zimbabwe and Malawi on account of their high proportion of meat to bone and their well-fleshed carcass (Maule, 1990). The Angoni cattle are highly susceptible to diseases such as theileriosis and trypanosomiasis (Billiouw et al., 1999). A report on agricultural development in Nigeria stressed that the future of beef production in tsetse areas must lie with improved lines of Zebu stock following control or eradication of tsetse flies (FAO, 1966).

1.3 Economic impact of diseases of cattle in sub-Saharan Africa

African animal trypanosomiasis constrains agricultural production in areas of Africa that hold the continent's greatest potential for expanded agricultural production (Swallow, 1999). The presence of the tsetse fly in one third of the African continent and the disease trypanosomiasis it transmits is considered the major barrier to the development of productive livestock (Vreysen, 2006). Despite the yearly administration of 35 million doses of trypanocidal drugs (at US$ 1 per dose), African farmers lose 3 million cattle every year to the disease and annual direct economic losses are estimated at US$ 600 to 1200 million

(Hursey and Slingenbergh, 1995.). Compared to animals kept in trypanosomiasis free areas, animals kept in areas of moderate risk of trypanosomiasis have lower calving rates, lower milk yields, higher rates of calf mortality, and require more frequent treatment with preventive and curative doses of trypanocidal drugs (Swallow, 1999). At the herd level, trypanosomiasis reduces milk offtake, live animal off-take and the work efficiency of oxen used for cultivation (Swallow, 1999). About 60 percent of the cattle at risk are not treated (Cattand et al., 2006). The potential benefits of improved trypanosomiasis control, in terms of meat and milk productivity alone, are $700 million per year in Africa. In 1999, the disease cost livestock producers and consumers in Africa an estimated $1.34 billion, without including indirect livestock benefits such as manure and traction (Kristjanson et al., 1999).

Trypanosomiasis is an important constraint, if not the most important constraint, to livestock and mixed crop-livestock farming in tropical Africa (Kristjanson et al., 1999; Swallow, 2000a). Animal trypanosomiasis constrains agricultural production, in particular, the use of draft power. Cattle infected with *Trypanosoma brucei brucei, Trypanosoma vivax,* or *Trypanosoma congolense* quickly succumb to a wasting form of anemia. In many areas with a high tsetse challenge, such as Central Africa, cattle are few or not present at all (Cattand et al., 2006). Constraints on draft power mean that farmers can till only small plots, making subsistence farmers extremely vulnerable to food shortages (Cattand et al., 2006). In spite of the importance of trypanosomiasis, economic instability and donor fatigue have led to a shortage of operational funds within the Government Veterinary Department, resulting in a rapid decline of resources available for tsetse and trypanosomiasis control in Zambia (Robinson et al., 2002).

Theileriosis, anaplasmosis, heartwater and babesiosis have been classified as the tick-borne diseases important to the livestock industry (Coetzer et al., 1994). East Coast Fever (ECF), affects millions of cattle in eastern and southern Africa (Norval et al., 1992). With over one million animals dying each year from ECF in sub-Saharan Africa, the disease represents one of the most important threats to livestock production in the tropics and is a major constraint on the livelihoods of millions of rural farmers (Minjauw and Mcleod, 2003; Rushton et al., 2002). Theileriosis, anaplasmosis, heartwater and babesiosis are present in Zambia (Pegram et al., 1986). Although the economic importance of East Coast Fever in Zambia is fully acknowledged, the frequency of occurrence of the disease may still be underestimated (Billiouw et al., 2002). In Zambia, East Coast Fever causes the largest number of cattle deaths in the country (Veterinary Department, Zambia, 1919 – 2006). Heartwater, caused by the rickettsial organism *Cowdria ruminantium*, is a serious constraint to livestock development in much of sub-Saharan Africa (Mukhebi et al., 1999). Heartwater is most severe in small ruminants, but also causes heavy losses in exotic cattle, which are more susceptible than indigenous breeds (Minjauw and Mcleod, 2003). In Zimbabwe, the estimated total annual national losses due to heartwater amounted to US$ 5.6 million over a 10 year period (Mukhebi et al., 1999). In Zambia, anaplasmosis counts amongst the most important of all the tick borne diseases (Makala et al., 2003). The global costs due to ticks and tick-borne diseases in cattle is estimated to be between US$ 13.9 and 18.7 billion (De Castro, 1997). Ticks are responsible for a variety of losses, caused by the direct effect of attachment ('tick-worry'), by the injection of toxins, or through the morbidity and mortality associated with the diseases they transmit (Minjauw and Mcleod, 2003). Economic losses caused by nematodes are in a variety of ways. Parasitism causes a reduction in food

intake and lower weight gains. Milk production can also be affected and mortality can occur in heavily parasitized animals (Rushton et al., 2002).

1.4 Major cattle diseases in Zambia

1.4.1 Trypanosomiasis

Trypanosomiasis is caused by several flagellated parasitic protozoa of the genus *trypanosoma*. The genus of trypanosomes is divided into two types (stercoraria and salivaria) depending on the development site in the vector tsetse fly (Hoare, 1972). Stercoraria trypanosomes are taken up by the vector in the blood meal and grow and divide in the insect's hindgut. Infection of the vertebrate host is by faecal contamination (e.g. *Trypanosoma theileri*). Salivaria trypanosomes (e.g. *Trypanosoma congolense, Trypanosoma vivax, Trypanosoma brucei*) develop in the mid gut and/or mouth parts of the vector and are injected via the salivary glands when the fly feeds (Leach and Roberts, 1981). Once inside the vertebrate host, the trypanosomes elude antibody attack by sporadically varying their surface glycoprotein, forcing the host to mount a new cycle of antibody production each time a new variant appears. In this way, the parasite manages to survive and increase its chances of transmission by tsetse or biting flies (Naessens, 2006).

Tsetse-transmitted livestock trypanosomiasis is a major constraint to livestock production in 37 countries of sub-Saharan Africa (Bossche et al., 2005; Kabayo, 2002; Maudlin, 2006; Ng'ayo et al., 2005; Swallow, 2000b). Transmission of trypanosomes is by tsetse flies of the *Glossina spp*. which are strictly blood feeders living exclusively in tropical Africa. The tsetse flies belong to the order Diptera, family Glossinidae and genus *Glossina* (Mulligan, 1970). There are thirty one species or subspecies, classified in three groups: *palpalis, morsitans* and *fusca*.

Fifteen species are in *fusca* group (subgenus *Austenina*), 9 in *palpalis* group (subgenus *Nemorhina*) and 7 in *morsitans* group (subgenus *Glossina sensu strict*) (Rogers and Robinson, 2004). Each species has distinct biological characteristics, but in general it may be said that the *palpalis* (riverine or lacustrine) group consists basically of the species living in the marginal areas of forests or near streams and rivers; the *fusca* or forest group consists of large sized species whose habitat is generally associated with equatorial forests; and the *morsitans* or savannah group consists mainly of species living in wooded savanna (Finelle, 1983).

Mechanical transmission of trypanosomiasis does occur (Andrews, 1927) and is effected by various blood-sucking insects such as flies of the family Tabanidae (horse flies) and *Stomoxys* spp. (Taylor, 1930). If a blood meal begins on an infected animal and ends on a healthy one, these insects may carry trypanosomes provided that the interval between the two meals is short. This form of transmission is the rule for *T. evansi,* but may also occur with trypanosomes habitually transmitted cyclically by *Glossina,* particularly *T. vivax* which may therefore be found in regions far from the *Glossina* distribution areas (Finelle, 1983). In the Eastern Province of Zambia, *Trypanosoma congolense (T. congolense)*, *Trypanosoma vivax* (*T. vivax)* and, to a lesser extent, *Trypanosoma brucei brucei (T. brucei brucei)* are the causal agents of trypanosomiasis in livestock (Masumu et al., 2006a; Masumu et al., 2006b; Naylor, 1971).

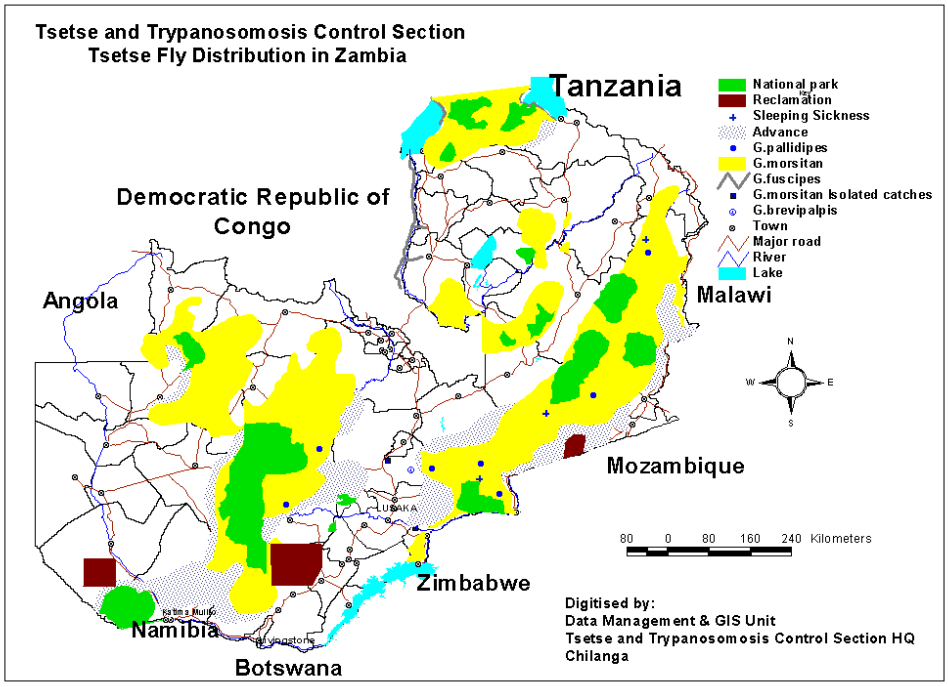

Figure 1. 2 Map of Zambia showing tsetse fly distribution and national parks: Source (DVLD, 2003).

In the plateau areas of Eastern Province of Zambia, trypanosomiasis is mainly spread by *Glossina morsitans (Glossina morsitans* and *Glossina centralis)* (Evison and Kathuria, 1982; Van den Bossche and De Deken, 2002). Other species of tsetse in the country are *Glossina pallidipes*, *Glossina fuscipes* and *Glossina brevipalpis* (Evison and Kathuria, 1982). The various tsetse flies distribution in Zambia are shown in Figure 1. 2. *Glossina morsitans* is the most widely distributed species covering 213,888 out of the 742,400 (28.8%) square kilometres of the country (Evison and Kathuria, 1982).

1.4.1.1 Human African Trypanosomiasis (HAT)

Human African trypanosomiasis (HAT) or sleeping sickness is a disease caused by infection with the protozoan *Trypanosoma brucei* (Bruce, 1895). *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* are two morphologically identical subspecies of *Trypanosoma brucei* (Bruce, 1915b; WHO, 1998). The two forms of the disease are transmitted by tsetse flies of the genus *Glossina* (order Diptera) and are restricted to sub-Saharan Africa. Both are fatal if left untreated (Chappuis et al., 2005). Generally, the disease is not found in urban areas, although some cases have been reported in suburban areas of some countries (WHO, 2010).

The clinical presentation in *T. b. rhodesiense* is more acute with clinical signs and symptoms developing within days following infection. The *T. b. gambiense* form of sleeping sickness is chronic, with months and sometimes years elapsing before the appearance of any obvious signs or symptoms (WHO, 1998). Around 300,000 to 500,000 people are currently infected and 100,000 deaths occur each year because of trypanosomiasis (Welburn et al., 2004; WHO, 2001). The disease has caused devastating epidemics during the past century but continues to be controlled by crisis management, using active case detection, treatment and vector control – activities that occur only during major epidemics; during the intervening periods, farmers and communities must fend for themselves (Maudlin et al., 2004).

Cattle are also an epidemiologically important reservoir for the human-infective parasite *T. b. rhodesiense* (Welburn et al., 2001). In Zambia, *T. b. rhodesiense* causes sleeping sickness mainly in the Luangwa valley in Eastern and Northern Provinces (Buyst, 1974; Foulkes, 1970; Rickman, 1974). It is estimated that around

500,000 people in Zambia are at risk of sleeping sickness due *T. b. rhodesiense* (WHO, 1998). Sleeping sickness is now rare in Zambia and can be missed or dismissed as retroviral disease, particularly in adults (Ngoma et al., 2004).

1.4.1.2. African Animal Trypanosomiasis (AAT)

Tsetse-transmitted trypanosomiasis is a classically acute or chronic disease that causes intermittent fever and is accompanied by anaemia, oedema, lacrimation, enlarged lymph nodes, abortion, decreased fertility, loss of appetite and weight, leading to early death in acute forms or to digestive and/or nervous signs with emaciation and eventually death in chronic forms (Office of International des Epizooties, 2008d).

African animal trypanosomiasis (AAT) or Nagana is caused by several species of trypanosomes mainly *Trypanosoma congolense, Trypanosoma vivax* and *Trypanosoma brucei* in cattle (Curson, 1927; Naessens, 2006). *Trypanosoma congolense* is found in most domestic mammals such as cattle, sheep, goats, horses, pigs, camels and dogs; and also in many wild animals. *Trypanosoma vivax* is a parasite of domestic and wild ruminants and of horses. *Trypanosoma simiae* is found mainly in domestic and wild pigs. *Trypanosoma brucei* is a parasite very close to *Trypanosoma b. gambiense* and *Trypanosoma b. rhodesiense,* which are the causes of human sleeping sickness *(Bruce, 1915a)*. It can infect practically all domestic and wild animals including dogs, horses, mules, donkeys, oxen, goats, sheep, monkeys, waterbucks and warthogs (Bruce, 1915a). In Zambia, it has been detected in hyena, waterbuck, lion and giraffe (Awan, 1979). *Trypanosoma evansi* is found in Africa only in the Sahara and Sahel regions where it is primarily a camel parasite, but it may be a parasite of horses, cattle and dogs as well.

Trypanosoma equiperdum is the causal agent of dourine, a contagious equine disease transmitted by coitus, which in Africa occurs only in the north African region and in South Africa (Finelle, 1983).

Severity of trypanosomiasis varies with species and age of the animal infected and the species of trypanosome involved. The incubation period is usually one to four weeks. Clinical signs of acute bovine trypanosomiasis include anaemia, weight loss, roughness of the hair coat, enlargement of peripheral lymph nodes and pyrexia (Eisler et al., 2004; Merck Veterinary Manual, 2007; Naessens, 2006). Cases progressing to a more chronic disease state may be characterized by anaemia, cachexia, poor productivity and infertility (Eisler et al., 2004).

It is well-established that anaemia is a characteristic feature of trypanosomiasis (Akinbamijo et al., 1998; Cherenet et al., 2006; Losos and Ikede, 1972). It is also known that voluntary feed intake by cattle during trypanosomiasis infections may be depressed, and this may compound the effects of the trypanosome infection on the haematological parameters of the host (Akinbamijo et al., 1998; Losos and Ikede, 1972). The clinical picture depends to some extent on the species of infecting trypanosomes and the geographical location (Murray, 1983). Sheep and goats infected with *T. brucei* species may show neurological signs like staggering and paralysis (Losos and Ikede, 1970.) Not all trypanosome strains living in the host's blood are invariably lethal, and the disease severity is strain dependent. In Europe and North America, the parasite *Trypanosoma theileri* occurs in a high percentage of otherwise healthy cattle and appears in blood cell cultures, but it does not cause illness (Verloo et al., 2000.).

In the Eastern Province of Zambia, *Trypanosoma congolense (T. congolense)*, *Trypanosoma vivax* (*T. vivax)* and, to a lesser extent, *Trypanosoma brucei brucei (T. brucei brucei)* are the causal agents of trypanosomiasis in livestock (Masumu et al., 2006a; Masumu et al., 2006b; Naylor, 1971). Previous studies in Petauke District established that 96% of trypanosomiasis infections are caused by *T. congolense* and the remainder are caused by *T. vivax* (2%) and *T. brucei.* (2%) (Sinyangwe et al., 2004). Zebu cattle found in Zambia are extremely susceptible to infections caused by *T. congolense* and *T. vivax* (Billiouw et al., 1999) .

1.4.1.3 Diagnosis of African Animal Trypanosomiasis

The primary reason for the diagnosis of bovine trypanosomiasis is for the appropriate application of therapeutic and sometimes prophylactic measures (Eisler et al., 2004). Identification of the causative agent can involve use of several parasite detection techniques, including the microscopic examination of the wet and stained thick or thin blood films (Office of International des Epizooties, 2008d). A highly specific and more sensitive test, used in an increasing number of laboratories, is the polymerase chain reaction (PCR), which can identify parasites at the genus, species or subspecies level, depending on the cases (Office of International des Epizooties, 2008d). Serological tests involve two trypanosomal antibody detection tests, the indirect fluorescent antibody test (IFAT) and the antibody-detection enzyme-linked immunosorbent assay (ELISA), are routinely used for the detection of antibodies in cattle. No vaccines are in use at the present time (Office of International des Epizooties, 2008d).

1.4.1.4 Treatment and control of African Animal Trypanosomiasis

Control strategies for bovine trypanosomiasis have been divided into three broad approaches (Barret J.C., 1997). The approaches are disease management using chemotherapy and chemoprophylaxis or use of trypanotolerant cattle, and vector control (Barret J.C., 1997; Grant, 2001).

The early treatment of trypanosomiasis was carried out using arsenic, its use was discontinued because of its toxicity (Anon., 1908). There are only a handful of drugs available today for treating African trypanosomiasis, most of which were discovered more than forty years ago (Wang, 1995). There are three categories of drugs available for the treatment of trypanosomiasis, these are isometamidium, diminazene and homidium salts (Geerts et al., 1998). Several drugs can be used for chemotherapy such as diminazene aceturate while isometamidium chloride is the chemoprophylactic drug that can be used to protect cattle from re-infection with trypanosomes for a period of up to 3 months (Geerts et al., 1998).

A number of tsetse control methods have been identified and tested in the field. These are bush clearance, slaughter of wildlife harbouring trypanosomes, application of insecticides on livestock, use of persistent and non-persistent chemicals (applied via aerial or ground spraying), fly traps and screens and the sterile insect technique (Hocking et al., 1963; Rushton et al., 2002).

Until the development of organochlorine insecticides (OCs) in the 1940s, the extermination of game (Andrews, 1927), and the destruction of bush were the principal means of tsetse control in Africa (Grant, 2001; Hocking et al., 1963). By the mid-1950s, the use of OCs as an effective and inexpensive approach to tsetse

control had virtually supplanted host and habitat destruction as the method of choice (Grant, 2001). Formulations of dichloro-diphenyl-trichloroethane (DDT) and dieldrin were applied to tsetse habitat either from the ground or from the air, and the persistence of the toxic molecules, was sufficient to kill many generations of flies (Grant, 2001). Aerial spraying has been used over more than 105 km^2 of savannah and riparian woodland in Zimbabwe, Zambia, Botswana, Somalia and Nigeria (Grant, 2001).

Targets and traps are not intended as a means to eradicate flies from an infested area, but to reduce populations of tsetse to levels that reduce the challenge or risks to humans and animals, and to prevent re-invasion of flies from a previously cleared area (Grant, 2001). The development and introduction of pyrethroids, insecticides of low mammalian toxicity, opened the way to their use in cattle dips. Because pyrethroids have acaricidal properties, protection of cattle from ticks, tsetse and other biting flies is achieved (Grant, 2001).

The sterile insect technique relies on the production of large numbers of the target insect in specialised production centers, the sterilization of the males (or sometimes both sexes), and the sustained and systematic release of the sterile males over the target area in numbers large enough in relation to the wild male population to outcompete them for wild females (Vreysen, 2006). Mating of the sterile insects with virgin, native females will result in no offspring. The release of sterile insects is however only effective when the target population density is low, it requires detailed knowledge on the biology and ecology of the target pest, and the insect should be amenable to mass rearing (Vreysen, 2006).

1.4.2 Tick-borne diseases

Tick-borne infections of livestock are widespread in Africa, where they present a greater constraint to livestock development particularly in cattle, than in any other region of the world (Perry and Young, 1995). The tick-borne diseases of livestock constitute a complex of several diseases whose etiological agents may be protozoal, rickettsial, bacterial or viral; their single common feature is that they can all be transmitted by ticks (Bram, 1983). Tick-borne diseases are present throughout the world, but are most numerous and exert their greatest impact in the tropical and subtropical regions. In many countries, they are the major health impediments to efficient livestock production (Bram, 1983). Theileriosis, anaplasmosis, heartwater and babesiosis have been classified as the tick-borne diseases important to the livestock industry (Coetzer et al., 1994).

1.4.3 Theileriosis

Theileriosis is caused by species of the protozoan *Theileria*, transmitted by ticks of the genera *Rhipicephalus, Hyalomma* and *Amblyomma. Theileriosis* is responsible for the most pathogenic tick-borne diseases of cattle with the most important and pathogenic being *Theileria parva* and *Theileria annulata* (Merck Veterinary Manual, 2007). *Theileria parva* which causes East Coast Fever (ECF), affects millions of cattle in eastern and southern Africa (Norval et al., 1992), while *T. annulata*, which causes tropical theileriosis, is widespread throughout the Mediterranean basin, the Middle East and Asia. Other species of *Theileria* are usually benign, although *T. mutans* can occasionally cause heavy losses (Minjauw and Mcleod, 2003).

1.4.3.1 East Coast Fever

East Coast fever (ECF) is a disease of cattle caused by the protozoan parasite *Theileria parva* (Jarrett et al., 1969a) which is transmitted by the three-host tick *Rhipicephalus appendiculatus*. *T. parva parva* (causing East Coast Fever), transmitted mainly between cattle, and *T. parva lawrencei* (causing Corridor disease), transmitted mainly from buffalo to cattle, are both highly pathogenic and can cause high levels of mortality, whereas *T. parva bovis* (causing January disease), transmitted between cattle, is less pathogenic (Merck Veterinary Manual, 2007; Uilenberg, 1999).

The incubation period for East Coast fever is 7 to 25 days (Siegel et al., 2006). The disease causes heavy mortality in exotic *Bos taurus* cattle of all breeds as well as in susceptible populations of indigenous *Bos indicus (Zebu)* breeds (Norval et al., 1991). The disease is characterized by high fever, swelling of the lymph nodes (Snodgrass et al., 1972), dyspnoea, and high mortality (Merck Veterinary Manual, 2007). In susceptible animals and in epidemic situations, mortality levels may reach 90% (Minjauw and Mcleod, 2003). Even in endemic areas, the mortality of *Theileria parva* infections is still estimated above 50% (Billiouw et al., 1999). Cattle recovered from East Coast Fever infection are unlikely to become ill with the disease a second time even when allowed to graze freely in enzootic areas (Nambota et al., 1994). In Zambia, East Coast Fever causes the largest number of cattle deaths in the country (Veterinary Department, Zambia, 1919 – 2006). In the Eastern Province of Zambia, the disease is responsible for killing a large number of cattle each year (Nambota et al., 1994; Turnbull, 1926).

1.4.4 Anaplasmosis

Anaplasmosis (formerly known as gall sickness) is an intra-erythrocytic rickettsial disease caused mainly by *Anaplasma marginale* and *Anaplasma centrale.* The disease occurs in tropical and subtropical regions worldwide and cattle, sheep, goats, buffalo, and some wild ruminants can be infected (Merck Veterinary Manual, 2007). Anaplasmosis is usually transmitted by ticks of the genus *Boophilus*, but it may also be transmitted mechanically by biting flies (e.g. Tabanidae and *Stomoxys*) (Minjauw and Mcleod, 2003).

Clinically the disease is characterized by a progressive anaemia (Bundza and Samagh, 1982), fever, weakness, constipation and the absence of haemoglobinuria. In severe cases these signs are accompanied by icterus, inappetence, depression, dehydration and laboured respiration. The syndrome is usually mild in animals less than one year old, but the morbidity is severe in adults (Boulanger et al., 1966) infected with *A. marginale* causing anaemia and up to 50% mortality (Minjauw and Mcleod, 2003). Chronic infection may persist for years without clinical signs (Bundza and Samagh, 1982; Minjauw and Mcleod, 2003).

Anaplasma spp. occur throughout Zambia but the disease appears to be in a state of endemic stability and outbreaks are rare among indigenous cattle (Jongejan et al., 1988). In Zambia, *Anaplasmosis* counts amongst the most important of all the tick borne diseases (Makala et al., 2003).

1.4.5 Cowdriosis

Cowdriosis or Heartwater is specific to cattle, sheep, goats and some wild ruminants, and is prevalent in much of Africa and the Caribbean. The causative organism is an intracellular parasite, previously known as *Cowdria ruminantium*. Molecular evidence led to reclassification of several organisms in the order Rickettsiales, and it is now classified as *Ehrlichia ruminantium* (Office of International des Epizooties, 2008b). It is transmitted by ticks of the genus *Amblyomma*. Heartwater is most severe in small ruminants, but also causes heavy losses in exotic cattle, which are more susceptible than indigenous breeds (Minjauw and Mcleod, 2003).

The clinical signs are dramatic in the per-acute and acute forms. In per-acute cases, animals develop fever, followed rapidly by hyperesthesia, lacrimation, and convulsions. In the acute form, animals show anorexia and nervous signs such as depression, a high-stepping stiff gait, exaggerated blinking of eyes, and chewing movements. Both forms terminate in prostration and convulsions. Diarrhoea is occasionally seen. In sub-acute cases, the signs are less marked, and Central Nervous System (CNS) involvement is inconsistent (Merck Veterinary Manual, 2007). The average natural incubation period is 2 to 3 weeks, but can vary from 10 days to 1 month (Office of International des Epizooties, 2008b). Heartwater occurs in domestic and wild ruminants in Zambia. The ticks, *Amblyomma hebraeum* and *A. variegatum* are the main vectors of heartwater in the agricultural areas of Zambia (Makala et al., 2003).

1.4.6 Babesiosis

Babesiosis is one of the most important diseases affecting cattle in tropical and subtropical regions of the world. It is caused by intra-erythrocytic protozoan parasites of the genus Babesia. Of the species affecting cattle, *Babesia bovis* and *B. bigemina* are widely distributed and of major importance in Africa, Asia, Australia, and Central and South America (Office of International des Epizooties, 2008a). The disease, which is transmitted by ticks of the genus *Boophilus*, affects a wide range of domestic and wild animals and occasionally humans (Merck Veterinary Manual, 2006). *Babesia bovis* is transmitted only by *B. microplus* whereas *B. bigemina* is readily transmitted by both *B. microplus* and *B. decoloratus* (Merck Veterinary Manual, 2006). *Babesia bigemina* has the widest distribution but generally, *B. bovis* is more pathogenic than *B. bigemina* (Office of International des Epizooties, 2008a).

Clinical signs of babesiosis include anaemia, fever (frequently 41°C or higher), jaundice, hemoglobinemia and hemoglobinuria (red water) occur in the final stages. CNS involvement may occcur with *B. bovis* infections. Late-term pregnant cows may abort, and bulls may undergo temporary infertility due to transient fever (Merck Veterinary Manual, 2006). Whereas high morbidity and mortalities are observed when mature animals are infected for the first time, calves generally exhibit a milder form of infection. In endemic areas, the existence of cattle herds often depends upon this generally mild natural pre-immunization (Levy et al., 1982). Animals that recover from Babesia infections are generally immune for life (Merck Veterinary Manual, 2006). Without treatment, mortality rates are very high ranging from 30% for *Babesia bigemina and* 70 to 80% for *B. bovis* (Minjauw and Mcleod, 2003).

In Zambia, Babesiosis due to *Babesia bigemina* is endemic, however outbreaks of the disease are rare in the country and this may be due to low levels of virulence (Rushton, 2001). Babesiosis due to *B. bovis* infection is not known to be a major disease in Zambia, but outbreaks occur occasionally in Eastern Province (Rushton, 2001). *Babesia bigemina* is distributed throughout the country while *Babesia bovis* is restricted to the eastern and north-eastern parts of the country (Jongejan et al., 1988). A similar distribution pattern of the respective tick vectors with *B. decoloratus* widely distributed throughout the country and *B. microplus* restricted principally to Eastern Province has been reported (Pegram et al., 1986).

1.4.7 Diagnosis of tick-borne diseases

Direct or indirect methods can be used for the diagnosis of tick-borne diseases. The direct method involves identifying the parasite in Giemsa-stained blood smears or lymph-node or other biopsy samples (Minjauw and Mcleod, 2003). It allows identification of all major tick-borne parasites and is the method of choice for the early treatment of their associated diseases. Indirect methods for the diagnosis of tick-borne diseases have traditionally been based on serology (Minjauw and Mcleod, 2003). The most widely used serological test is the indirect fluorescent antibody test (IFAT), but this is time-consuming and labour intensive. Serological tests based on monoclonal antibodies, such as competitive enzyme-linked immunosorbent assays (ELISAs) and Western blotting are usually more specific (Minjauw and Mcleod, 2003). Other tests, such as those involving nucleic acid probes or the polymerase chain reaction (PCR), require laboratories of the very highest standard (Minjauw and Mcleod, 2003). Diagnosis of tick-

borne diseases is also based on clinical signs, knowledge of disease and vector distribution (Office of International des Epizooties, 2008c).

1.4.8 Treatment and control of tick-borne diseases

Efficacy of East Coast fever chemotherapy depends on early and fast diagnosis (Billiouw et al., 1999). Several drugs such as parvaquone (Clexon®), buparvaquone (Butalex®) and halofuginone lactate (Terit®) are available for the treatment of East Coast fever (Minjauw and Mcleod, 2003). However, treatment must be initiated at the onset of clinical signs to be effective. These drugs are expensive and may be cost-prohibitive. Tetracyclines also can be used to treat animals with East Coast fever (Pegram et al., 1996), but as with the other drugs, treatment must be initiated early at the onset of clinical signs (Merck Veterinary Manual, 2007; Siegel et al., 2006).

Anaplasmosis can be effectively treated, especially in the early stages of infection, with tetracycline antibiotics (oxytetracyclines or chlortetracycline) and with imidocarb dipropionate (Minjauw and Mcleod, 2003). *Erlichia ruminantium* infections are generally treated with tetracycline antibiotics, which can also be used prophylactically during peak periods of *Amblyomma* activity. A slow-release formulation of doxycycline hydrochloride is now available in the form of 75-mg tablets for subcutaneous implantation, making this method of control even more effective (Minjauw and Mcleod, 2003). A variety of drugs have been used to treat babesiosis in the past, but only diminazene aceturate and imidocarb dipropionate (also used to treat anaplasmosis) are still in common use (Merck Veterinary Manual, 2007). Long-acting tetracycline may reduce the severity of

babesiosis if treatment begins before or soon after infection (Merck Veterinary Manual, 2006).

The most common method used to prevent East Coast fever is to infect and treat susceptible animals (Jarrett et al., 1969b). Animals are inoculated with an extremely high dose of sporozoites harvested from ticks and then treated with one of the drugs mentioned previously. Since cross protection does not occur, the inoculum must contain multiple *Theileria* species/ strains. Immunity conferred from this form of immunization lasts for approximately 3½ years. Control of East Coast fever and other tick-borne diseases is based on a multifaceted approach including pasture management, herd-selection of resistant animals, strategic tick control, immunization and treatment of clinical cases (Minjauw and Mcleod, 2003; Siegel et al., 2006).

1.4.9 Pasture-transmitted diseases

Pasture-transmitted diseases are transmitted directly from vertebrate host to vertebrate host via the pasture without the need of a vector. The important pasture transmitted diseases are species of nematodes found in Africa and affecting ruminants and include *Haemonchus placei, Trichostrongylus axei, Cooperia pectinata., Cooperia punctata, Bunostomum phlebotomum* and *Oesophagostomum radiatum* (Sauvage et al., 1974; Waruiru et al., 2000; Waruiru et al., 1998). Trematodes or flukes are also pasture transmitted diseases and important trematodes in the tropics include *Fasciola* spp. (Rushton et al., 2002).

The clinical signs associated with pasture transmitted diseases are shared by many diseases and conditions; however, presumptive diagnosis based on signs, grazing history, and season is often justified. Infection usually can be confirmed

by demonstrating nematode eggs or tapeworm segments on faecal examination (Merck Veterinary Manual, 2007). The advent of safe and effective broad-spectrum anthelmintics has largely reduced the need for differentiating the genera and species of these parasites (Merck Veterinary Manual, 2007).

The planning of an effective strategy for the control of pasture transmitted diseases requires an understanding of the ecology of the parasites and their geographic and seasonal distribution and prevalence (Rushton et al., 2002). The influence of management practices will also need to be considered and, perhaps of greatest importance to the farmers, the economic impact of the control (Rushton et al., 2002). The measures which are used to control gastrointestinal parasites include use of anthelmintic drugs, vaccines, use of resistant animals and improved management practices (Rushton et al., 2002).

Control strategies that involve the control of grazing are difficult for poor farmers to implement. Livestock will often be grazed on communal land and the resources and labour necessary to restrict grazing are likely to be in short supply (Rushton et al., 2002). Effective worm control cannot always be achieved by drugs alone; however, anthelmintics play an important role (Merck Veterinary Manual, 2007).

1.5 Anaemia as an indicator of diseases

Many diseases such as trypanosomiasis, east coast fever, anaplasmosis, babesiosis, heartwater and helminths have anaemia as an important presenting clinical sign (Bundza and Samagh, 1982; Eisler et al., 2004; Hendrickx et al., 2004; McCrorie et al., 1980; Merck Veterinary Manual, 2007; Minjauw and Mcleod, 2003).

In Burkina Faso, the link between trypanosomiasis and the occurrence and presence of anaemia in field situations has been demonstrated (Hendrickx et al., 2004). Anaemia is one of the most important indicators of trypanosomiasis in cattle (Stephen, 1986) and is an index of animal health in bovine trypanosomiasis (Mahamaa et al., 2004). As other diseases may also cause anaemia, anaemia may be used as a measure of the general health status of surveyed herds (Hendrickx et al., 2004). Trypanosomiasis infection, regardless of species, results in anaemia which is evidenced by a significant decrease in the packed red blood cell volume (PCV) of the infected animal (Cherenet et al., 2006). A strong correlation between the low cattle numbers and the PCV which is an indicator of anaemia and animal health status was shown in West Africa (Hendrickx et al., 2004). The level of anaemia or the PCV usually gives a reliable indication of the disease status and productive performance of an infected animal (Trail et al., 1990; Trail et al., 1993). The presence, absence and degree of anaemia are therefore important diagnostic criteria for the aforementioned diseases (de Bont, 1998) with anaemic animals being of poor health.

1.5.1 Anaemia and polycythaemia in livestock

Anaemia is defined as an absolute decrease in the red cell mass as measured by red blood count, haemoglobin concentration, and PCV. It can develop from loss, destruction, or lack of production of red blood cells (Merck Veterinary Manual, 2007). Anaemia is classified as regenerative or non-regenerative. In a regenerative anaemia (also called responsive anaemias), the bone marrow responds appropriately to the decreased red cell mass by increasing red blood cells (RBC) production and releasing reticulocytes (e.g. haemorrhagic and

haemolytic anaemia) (Merck Veterinary Manual, 2007). This indicates that the erythropoietic response mechanisms are intact and the erythropoietic tissues are healthy (Tyler and Cowell, 1996).

In a non-regenerative anaemia, the bone marrow responds inadequately to the increased need for red blood cells (E.g. nutritional deficiencies, primary bone marrow disease, renal disease, anaemia of chronic disease). Anaemias due to haemorrhage or haemolysis are usually regenerative. Anaemias that are caused by decreased erythropoietin or an abnormality in the bone marrow are non-regenerative (Merck Veterinary Manual, 2007). Inadequately regenerative anaemias occur when a condition causes haemolysis and/or haemorrhage and concurrent partial suppression of erythropoiesis or when conditions that incompletely suppress erythropoiesis occur concurrently with conditions that cause haemolysis and/or haemorrhage.

Polycythaemia is a relative or absolute increase in the number of circulating red blood cells resulting in an increased packed cell volume, red blood cell count, and haemoglobin concentration (Merck Veterinary Manual, 2007). Relative polycythaemia can be caused by any mechanism that results in haemoconcentration, such as dehydration from vomiting or diarrhoea, or in a fluid shift from the intravascular to the extravascular space due to increased vascular permeability. A loss of plasma volume will result in an apparent increase in red blood cell numbers (Merck Veterinary Manual, 2007).

Absolute Polycythaemia results in a real increase in red blood cell numbers as a result of increased production. Absolute polycythaemia may be primary or secondary. Primary polycythaemia, or polycythaemia rubra vera, is a myelo-

proliferative disease of unknown cause. It has been reported in dogs, cats, and cattle (Merck Veterinary Manual, 2007). Red blood cell production is dramatically increased, and serum erythropoietin levels are low or below normal. In secondary polycythaemia, red blood cell production increases in response to increased erythropoietin levels (Chevalier et al., 2003; Cork and Halliwell, 2002).

There are various theories on the pathogenesis of anaemia caused by trypanosomiasis (Uilenberg, 1998). In the early stages of the disease, it is believed to be caused in part by phagocytosis of red cells (Uilenberg, 1998). The red cells become coated with material from lysed trypanosomes which tricks the phagocytes into mistaking them for foreign invaders and removing them (auto-immunity) (Uilenberg, 1998). It is possible that the anaemia caused by phagocytosis is increased by toxic substances emanating from the trypanosomes which destroy red cells directly by lysis (haemolysis) (Uilenberg, 1998). The haemopoietic system tries to compensate for the loss of erythrocytes by increasing its activity but, later, in the chronic stages of trypanosomiasis, other toxins from the parasites have a depressing effect on the haemopoietic system, and the host is unable to produce as many red cells as are removed and now they are removed even faster because of auto-immunity and haemolysis (Uilenberg, 1998).

1.5.2 Detection of Anaemia

The oxygen-carrying capacity of blood largely depends on its haemoglobin concentration. When anaemia is suspected, the determination of packed cell volume and/or haemoglobin concentration can document its presence (Timan et al., 2004). Haemoglobin measurement is essential for diagnosis of anaemia, for

assessing its severity and for management of patients (Merck Veterinary Manual, 2007).

The standard method for measuring haemoglobin concentration requires conversion of haemoglobin in a sample of blood to cyanmethaemoglobin, followed by photometric quantification of the cyanmethaemoglobin. This method is technically complex; therefore, samples must typically be submitted to a well-equipped laboratory for analysis (Cork and Halliwell, 2002). The microhaematocrit method is also often used to estimate the PCV, but measuring it requires the use of a centrifuge and it takes several minutes (Cork and Halliwell, 2002). Alternatively, the packed cell volume can be roughly estimated by multiplying haemoglobin concentration (g/dL) by 3, but this requires first measuring the haemoglobin concentration (Cork and Halliwell, 2002). In the context of delivery of veterinary services in developing countries such as many in sub-Saharan Africa, PCV determination using the microhaematocrit method requires expensive portable generators and centrifuges as well as vehicles to transport them, which are rarely affordable and sustainable by the extension agents (Magona et al., 2004a).

The haemoglobinometer is a portable, rapid, and technically simple method for measuring haemoglobin concentration in the field (Radtke et al., 2004). In human medicine, portable photometers are increasingly used for haemoglobin screening in blood centres (Schenck et al., 1986). The Hemocue 201+ which is a hand-held haemoglobinometer and measures haemoglobin at two wavelengths as azide methaemoglobin, without dilution (Hemocue, 2008). The azide methaemoglobin reaction involves the erythrocyte membranes being disintegrated by sodium deoxycholate, thereby releasing the haemoglobin. Sodium nitrate then converts

the haemoglobin iron from the ferrous to the ferric state to form methaemoglobin, which then combines with azide to form azide methaemoglobin (Hemocue, 2008; Schenck et al., 1986).

This method is based on an optical measuring microcuvette of a small volume (10µl) and short light path (0.13 mm distance between the parallel walls of the optical window). Dry reagents are deposited on the inner wall of the microcuvette cavity, and the blood sample, drawn into the cavity by capillary action, is mixed with the reagents spontaneously (Schenck et al., 1986). The microcuvette is then placed in the Hemocue photometer in which its absorbance is measured at 570 and 880nm (Schenck et al., 1986). The 570 nm wavelength is used for determining haemoglobin concentration and 880 nm to correct for any turbidity in the sample. The conjugation reaction for the azide moiety is 4 times as fast as the conjugation reaction for cyanide, thus substantially reducing the time required for sample analysis. The instrument calculates the concentration of the haemoglobin in the sample and displays the result within 60 seconds (Schenck et al., 1986). The Hemocue system is suitable for field surveys and correlation with measurements from an automated system is generally good (Gies et al., 2003). Haemocue readings have a variation of ±0.3 g/dl (Hemocue, 2008). The Hemocue is portable, easy to use (Schenck et al., 1986), has no inter-observer error, precise and accurate, but is expensive (Magona et al., 2004b). In cattle, the normal range of haemoglobin is 8 – 15 g/dl (Schalm et al., 1975).

1.6 Assessment of cattle movement behaviour

The behaviour of an animal can be a clear indicator of its physiological state (Frost et al., 1997). A diseased animal may be more, or less, active than a healthy one while animals suffering from a cold environment may huddle together for

warmth (Frost et al., 1997). An animal's activity level may also be linked to its stage in the reproductive cycle (Frost et al., 1997).

1.6.1 Challenges of observing accurate cattle movement behaviour in field conditions

Observing accurate cattle movement behaviour in field conditions presents a number of challenges. One of the challenges is that natural movement patterns can be influenced by the presence of human observers. Evaluation of normal cattle behaviour patterns and individual health status is difficult to accomplish due to the animal's response to human interaction and presence (Robert et al., 2009). Monitoring and recording animal movement activity is time consuming, subjective and laborious (Blackie et al., 2006; Frost et al., 1997).

The disadvantages of the stockman being in total control of the planning, monitoring and running of the livestock unit include: lack of time or opportunity to observe each animal thoroughly; infrequent visits for observation, often for only a few minutes each day; visits disturb the animals, so the animals' behaviour may not be typical of the diurnal rhythms; observations are subjective (Frost et al., 1997). The stockman is able to collect and assimilate only fragmented pieces of information on which to make his management decisions (Frost et al., 1997).

1.6.2 Methods of assessing cattle movement behaviour

Developments in sensor technology will make available increasing amounts of information relevant to monitoring animals and their environment, and hence their production, growth and health (Frost et al., 1997). Traditional visual

observational techniques of cattle movement behaviour have been employed in the past (Kabuga et al., 1991; Mazrier et al., 2006; Rae et al., 1999; Solano et al., 2005). Other methods such as constant video surveillance allows non-invasive behaviour monitoring, but logging the movement patterns on individual animals over long periods of time is often cost prohibitive and labor intensive (Robert et al., 2009). Global Positioning System (GPS) collars have also been used to measure cattle movement behaviour (Brosh et al., 2006).

1.6.3 Assessment of cattle movement behaviour using motion sensors

Cattle movement behaviour is potentially a valuable indicator of health and well-being (Robert et al., 2009). A remote system could augment the ability of researchers, and eventually cattle producers, to monitor changes in cattle behaviour (Robert et al., 2009). Motion sensors offer the potential to remotely monitor animal behaviour and document the percent of time an animal spends involved in a particular activity (Blackie et al., 2006). Motion sensors have been found to be accurate when compared to video analysis when classifying cattle behaviour into one of three activities of standing, walking or lying down (Robert et al., 2009). Motion sensors provide an objective, non-invasive measure of activity that may be linked to specific animal health or performance outcomes. The technology can be used immediately in research endeavors where the objective is to determine differences in cattle behaviour between treatment or management groups (Robert et al., 2009).

The development of an objective system for monitoring and assessing activity of cattle to identify animals at risk for disease could prove useful in alleviating health and economic costs associated with illness (Robert et al., 2009). Utilization

of technology to automatically record behaviour allows for collection of objective values. Motion sensors may offer a viable system to monitor changes in cattle behaviour, which in turn could be used to document animal wellness (Robert et al., 2009).

Cattle movement activity can be measured from a motion sensor attached to a leg band or a neck chain of an animal (Edwards and Tozer, 2004). Changes in activity can be viewed with ease and diagnosis made for animals with decreased activity. There has been limited research to show the effects of animal health on daily walking activity (Edwards and Tozer, 2004).

Motion sensors are an efficient way of automatically monitoring animal activity over a long period of time (Blackie et al., 2006). IceTag™ activity monitors are motion sensors designed specifically for use on animals (IceRobotics, 2007). IceTag™ motion sensors use an electronic accelerometer to automatically determine the percentage of time that the animal spends lying down, standing or walking. Data is captured 8 times per second and algorithms are used to determine the average per cent of time allocated to each of these behaviours (Blackie et al., 2006; IceRobotics, 2007).

The device has a tough plastic housing designed to withstand farm environments and a simple strap for easy attachment and removal. It is attached to the hind-leg of the animal at the metatarsus proximal to the fetlock joint. The IceTag™ motion sensors can store up to 30 days of data between downloads while also giving the time sequence. The downloads are via a simple USB cable using a windows application (Roelofs et al., 2005; Senger, 1994).

It has been shown that motion sensors can be used to detect oestrus accurately in dairy cattle (through the increase in number of steps around oestrus) (Arney et al., 1994; Moore and Spahr, 1991) and appear to be a promising tool for prediction of ovulation and hence could be a tool for improving fertilization rates (Senger, 1994). The efficiency of motion sensor technology compared with that of visual observation for oestrus detection is quite variable and ranges from 60 to 100% efficiency, depending on the study (Mazrier et al., 2006). In addition to the early detection of oestrus, the motion sensor is also useful for detecting lameness although further studies are needed to improve the motion sensor's capability to predict the onset of lameness (Tyler and Cowell, 1996).

1.7 Thesis objective

1.7.1 Overall objective

The overall objective of this work was to study the movement behaviour of traditionally managed cattle in the Eastern Province of Zambia using two-dimensional motion sensors. The impact of a triple co-administered broad-spectrum treatment on cattle movement behaviour was also evaluated. It is hypothesised that objectively verifiable indicators for disease such as haemoglobin influence the cattle movement behaviour of cattle in the Eastern Province, Zambia. It is also hypothesised that administering a broad-spectrum treatment to cattle will affect their movement behaviour. To test these hypotheses, various activities were carried out as outlined in the specific objectives below.

1.7.2 Specific objectives

- Determine whether Icetag™ two-dimensional motion sensors are suitable devices for investigating and quantifying the movement behaviour of traditionally managed cattle in Petauke district, Eastern Province of Zambia.
- Determine whether there are any differences in cattle movement behaviour between two areas within the study site.
- Determine whether there are any differences in cattle movement behaviour between animals with different haemoglobin levels.
- Determine the effect of a triple co-administered broad-spectrum treatment on cattle movement behaviour.

1.7.3 Overall design of the studies presented in this thesis

The study design consisted of several parts and aimed at achieving the overall and specific objectives. The study design consisted of the following activities:

- Collecting historical data on trypanosomiasis and tick-borne diseases for Zambia.
- Assessment of Petauke demographic and livestock management characteristics using a standardised questionnaire.
- Conducting a cattle movement behaviour study using two-dimensional motion sensors in Makale and Kasero Veterinary Camps, Petauke.
- Assessing the impact of a triple co-administered broad-spectrum treatment on cattle movement behaviour using two-dimensional motion sensors in Makale Veterinary Camp.

Complete details the study design are given in the Materials and Methods chapter (2) and other respective chapters.

1.7.4 Importance of the studies presented in this thesis

The study results will provide an understanding of the movement behaviour of traditionally managed cattle in Sub-Saharan Africa. The findings will also show how the movement behaviour of animals will change in relation to an objectively verifiable indicator for disease such as haemoglobin. This information can help stakeholders in making management and disease control decisions that maximise livestock production benefits from the limited resources. Additionally, the study results will provide baseline data for Zambia on which future research on cattle movement behaviour can be based.

CHAPTER 2

General Materials and Methods

2.1 Field methods

The general materials and methods that were used during the study and which are common to all chapters are dealt with here. Materials and methods specific to chapters are contained within their respective chapters.

2.1.1 Programme Sensitisation

Prior to starting the field work, a number of sensitisation meetings were held with government officials from the Department of Veterinary and Livestock Development in their Lusaka headquarters; Chipata, Eastern province headquarters and the Petauke district offices. Other meetings were conducted in the proposed study areas of Petauke district with cattle owners and village leaders. The meetings aimed at informing stakeholders about the programme and seeking their support and cooperation.

2.1.2 Sampling frame

The numbers of animals sampled were specific to each chapter and are discussed in their respective chapters.

2.1.3 Clinical examinations

Clinical examinations were conducted in the field on all cattle that were presented for the study (Figure 2. 1). Before any clinical examination could be carried out, all animals that did not have ear tags were tagged for ease of identification. The owner's consent was sought before tagging of the animals was carried out. Those animals that already had tags were not tagged again but had their existing tag colours and numbers used for identification. The examinations

were carried out by a veterinarian or veterinary assistant. Clinical parameters were recorded onto the field record sheet (Table 2.1). The clinical parameters were systematically recorded and included measurements of rectal temperature, haemoglobin levels, colour of mucous membranes, size of lymph nodes, and presence of diarrhoea, presence of lacrimation and body condition scores. In addition, the examining veterinarian and cattle owner were asked to assess the health status of each of the animals examined.

Figure 2. 1 Animals awaiting clinical examination at a crushpen in Makale, Petauke District

Table 2. 1 Field record sheet

Date:…… District: Petauke **Veterinary camp:…….……..…….**

Village:……. GPS Coordinates:…….…..

Name of owner:…… Name of VA/VO:…….……..…..

Tag No	Haemo-globin, g/dl	Sex	Age	MM: N, P, PP by VA/VO	Lymph Node, Size N/E	Skin Lexs, 0,1,2	Skin Coat, N, S	Diarrhoea, 0, 1, 2	Discharge Site and Severity	Health status by owner HH, H, Sk, SSk	Weight, Kg	Body condition score F+,F,F-M+,M,M-L+,L,L-	Rectal Temp °C

MM = Mucous Membranes, N=Normal, P=Pale, PP = Very Pale, E = Enlarged, S = Starring, HH = Very Healthy, H = Healthy, Sk = Sick, SSk = Very Sick,
Skin Lexs = Skin Lesions , Diarrhoea, 0 = none, 1 = slight, 2 = heavy
Body condition scores are F [fat], M [medium] and L [lean] subdivided into three categories with scores from 1 to 9. F+ [9], F[8], F-[7]; M+[6], M[5], M-[4]; L+[3], L[2] and L-[1]
VA = Veterinary Assistant, VO = Veterinary Officer

2.1.3.1 Body condition scoring

Nicholson *et al* (1986) provides guidelines on the body condition scoring of *Bos indicus* (Zebu) cattle which may be assessed visually and expressed as a condition score. The extent to which fat is stored or muscle mass has diminished on an animal is due to the nutritional plane to which that animal has been exposed over a reasonable length of time. Measuring changes in weight per se does not reflect an animal's condition due to the different skeletal sizes of animals. The large variations in gross live-weight may also occur as a result of changes in the gut and bladder fill, pregnancy, parturition and tissue hydration (Nicholson and Butterworth, 1986).

In the body condition score system designed by Nicholson and Butterworth (1986), nine scores are used in which the three main conditions - (fat [F], medium [M] and lean [L]) are subdivided into three categories. The scores are abbreviated as F+, F, F-; M+, M, M-; L+, L and L-. Each scoring is given a number from 1 (L-) to 9 (F+). In a borderline case a half point maybe added to the lower score, so that a cow described as M-/L+ would be scored as 3.5. However, this was rarely if ever found to be necessary. The score of an animal depends on the visibility of the anatomical parts, and the flesh and fat cover at these points. There is a highly positive correlation between body condition and resource availability represented as finance, management skill and grazing availability (Reed et al., 1974). Scoring has however, been found to be least reliable in the case of young calves and weaners, as growing animals tend not to have heavy deposits of fat. The purpose of giving numbers to the conditions is to allow statistical analysis of the data and to facilitate codification on computer files (Nicholson and

Butterworth, 1986). Condition scoring in the field was performed as described above with cattle being scored early in the morning and having had no access to food or water overnight as recommended.

2.1.3.2 Weighing of animals

All cattle had their body weights estimated using a weigh band (CEVA, Sante Animale, France). In all cases, the weighing of the animal took place in a crushpen or kraal with the animal standing up. The weigh band was placed behind the hump and immediately behind the front legs under the chest to obtain a reading. The weights of the animals were used for administration of drugs and health evaluation of the animals.

2.1.3.3 Condition of coat

The body coat condition of the animals was evaluated and recorded. Each individual animal was inspected to assess the condition of the coat. Assessment of the coat determined whether the hair of the animal was normal, rough or starring. The coat was either recorded as normal or starring. A starring coat was recorded when it was rough, standing and not shiny.

2.1.3.4 Condition of lymph nodes

The lymph nodes were palpated to establish whether they were normal size or enlarged. The superficial lymph nodes examined were the pre-scapular, parotid, submandibular and pre-femoral.

2.1.3.5 Diarrhoea

If the animal had diarrhoea, this was recorded on the field record sheet. Animals that had diarrhoea were soiled with watery faeces below and around the anus and on the hind legs.

2.1.3.6 Lacrimation

Animals were classified as having lacrimation when they had a discharge from either or both eyes. This was recorded as a yes on the record sheet while a no meant there was no discharge from either eye.

2.1.3.7 Rectal temperature

Rectal temperature was recorded using a digital thermometer during the course of the clinical examination.

2.1.3.8 Mucous membranes

The visible mucous membranes were examined to assess whether the animal was anaemic, had petechial haemorrhages or was jaundiced. The visible mucous membranes examined were the oral, conjuntival and vulval mucous membranes.

2.1.3.9 Skin lesions

Each animal was inspected cranio-caudally for any skin lesions on the body. If present, the severity of the lesions was graded.

2.1.4 Blood sample collection

All cattle had blood collected from them after being adequately restrained. Collection of blood was done by pricking the bovine ear vein using a 16G needle into microcuvettes and used for measurement of haemoglobin (Hb) using the Hemocue Hb 201+ (HemoCue AB, Ängelholm, Sweden). Blood was also collected using capillary tubes onto Whatman FTA cards (Whatman Inc., NJ, USA) for laboratory Polymerase Chain Reaction (PCR) analysis.

2.1.4.1 Haemoglobin Measurement

Figure 2. 2 HemoCue Hb 201+ analyser (Source: HemoCue AB, Ängelholm, Sweden).

The haemoglobin measurements were done using the Hemocue Hb 201+ (HemoCue AB, Ängelholm, Sweden). Blood collected from the ear vein was used to fill the microcuvette in one continuous process. The excess blood on the outside of the microcuvette tip was wiped off making sure that no blood was drawn out of the microcuvette. Additional care had to be taken to make sure that there were no air bubbles in the microcuvette or a new sample had to be taken. The filled microcuvette was then placed in the cuvette holder of the HemoCue Hb 201+ (Figure 2. 2) within ten minutes after filling the microcuvette.

The haemoglobin value of the blood was then displayed in g/dl after 15 to 60 seconds of initiating the reading. The haemoglobin value was noted down on the field data record sheet and then later transferred to an excel spreadsheet for storage and analysis.

2.1.4.2 Collection of blood onto FTA cards

Figure 2. 3 An FTA card with blood spots after being punched

Blood was transferred onto FTA® cards (Whatman International LTD, UK) using capillary tubes. Application of the blood onto the cards was done so that there was no saturation and no contact of the capillary tube with the FTA® cards to avoid contamination. Each FTA card holds up to four blood spots and identification details of area, date and tag number were entered on each card to identify the animals sampled (Figure 2. 3). Once four blood spots were placed on the FTA card it was dried overnight in dust free conditions before being packed

in sealable foil packs with silica desiccants to protect against light and moisture. The sealed packs were then stored in a cool dry place at ambient temperature for later transport to the University of Edinburgh (United Kingdom).

2.1.5 Two-dimensional motion sensor data collection

Figure 2. 4 Cattle with an ear tag and motion sensor attached to the hind leg

The two-dimensional (2D) motion sensor (pedometers) uses electronic accelerometers orientated in two orthogonal (perpendicular) dimensions to record the lying, standing and walking activity of animals. The motion sensors used in this study are the IceTag™ activity monitors (IceRobotics, UK).

Prior to attaching motion sensors to their animals, the consent of the farmers was sought after explaining the purpose of the motion sensors and that they posed no harm to animals or humans. The selection of animals for motion sensor attachment was based on several factors. Animals that had motion sensors

attached were in co-grazing pairs. The pairs were of the same sex and had as close as possible the same age. The age was calculated from the estimated date of birth given by the cattle owner. Selected animals were also required not to be draft animals. Other criteria used in the selection of cattle for motion sensor attachment will be discussed in relevant chapters. Farmers were asked to be vigilant by reporting any motion sensor that fell off the animals noting the day and time. The motion sensors were placed on the animals' hind leg (Figure 2. 4), to continuously measure and record its activity for two to three weeks depending on the study. The 2D-motion sensors (pedometers) were attached to the hind limbs of cattle using a Velcro strap (Figure 2. 5).

Figure 2. 5 Attachment of a motion sensor to the hind leg of an animal using Velcro strap.

2.1.6 Geo-referencing of sample collection sites

All sample collection sites were geo-referenced using a handheld global positioning system (GPS) device, Etrex 12 channel GPS (Garmin, USA). The GPS recorded the specific longitude and latitude coordinates including the altitude above sea level. The GPS coordinates were used for mapping of the sampling sites and crush pens and also areas where cattle had motion sensors attached.

2.1.7 Data storage and analysis

Data obtained on cattle clinical parameters, cattle owner details, haemoglobin data, treatments administered and laboratory results were entered and stored in Microsoft Excel (2003). Motion sensor data was downloaded from the units in the field onto a laptop computer via a USB cable using the IceTag Analyser software (Research Version 2.003) and saved as ".csv" files before being exported to Microsoft Excel (2003). Depending on the applications required, the software that was used for data analysis was Minitab version 14 (Minitab Inc.), Microsoft Excel (2003) and IceTag Analyser software (Research Version 2.003). Calculations of summary descriptive statistics were carried out using Minitab 14. Microsoft Excel (2003) and Minitab 14 were used to draw graphs. Statistical comparisons by proportion were done by Chi-square tests using Minitab 14. The comparison of mean haemoglobin concentration values was done using 2-sample t-tests with Minitab 14. A 2-sample Wilcoxon rank sum test was used to test the equality of two population medians.

2.2 Laboratory Methods

2.2.1 Sample collection

The collection methods of blood samples that were used in the laboratory are covered in the field methods part of this chapter (Section 2.1).

2.2.2 FTA cards

FTA (Flinders Technology Associates) matrix cards were developed by Whatman and contain chemicals that lyse cells, denature proteins and protect nucleic acids from nucleases, oxidative, and UV damage. FTA cards rapidly inactivate organisms, including blood-borne pathogens, and prevent the growth of bacteria and other microorganisms. Cell membranes and organelles are lysed and the released nucleic acids are entrapped in the fibres of the matrix. The nucleic acids remain immobilized and are stabilized for transport, immediate processing or long-term room temperature storage. Genomic DNA stored on FTA cards at room temperature for over 17 years has been successfully amplified by PCR (www.whatman.com). FTA cards can be sent via ordinary mail packages without a hazardous warning because any pathogenic organisms that are in the sample are inactivated upon contact with the card matrix. The FTA cards also remove the necessity for temperature control when transporting samples (www.whatman.com).

2.2.2.1 FTA card processing

Using a 2 mm handheld punch device, a total of five discs per sample were made (Picozzi et al., 2002). Each set of samples was put in separate 1.5 ml Eppendorf tubes that had been earlier labelled correspondingly with tag numbers. Between punches, the handheld punch was cleaned by cutting two punches out of the clean filter paper. After punching the required samples the punch was cleaned by multiple punching of the clean filter paper and put under UV light for 15 minutes prior to next use.

2.2.2.2 Sample washing

The samples in the Eppendorf tubes were washed in order to free them of blood intrinsic PCR inhibitors such as haem. Each sample in the 1.5ml Eppendorf tubes was washed twice for 15 minutes with constant agitation in 1 ml FTA purification reagent (Whatman® International Ltd, UK). This was then followed by two 1 ml washes lasting 15 minutes each of x1 concentrated Tris-EDTA (TE) (10 mM Tris HCl pH8; 1 mM EDTA) (Sigma-Aldrich Company, UK). The TE buffer wash was necessary as it removed the FTA purification reagent which also inhibits PCR. To prevent contamination, care was taken not to touch sample tubes with the pipettes during the washing process.

2.2.2.3 DNA Elution with Chelex Resin

The Chelex Resin used was the Chelex 100 which has a pH 11 in aqueous solution and in addition to removing PCR inhibitors it also aids the dissociation of DNA into its component single strands thus facilitating PCR amplification. The washed samples were transferred to open PCR tubes (Alpha Laboratories, UK) and dried at 37°C for 30 minutes in an oven. Each set of samples was placed

in a single PCR tube and correspondingly labelled. After drying, 5 % (w/v) aqueous suspension Chelex ® 100 resin (sodium form, 50-100 dry mesh; Sigma-Aldrich, Dorset, UK) was added to each sample and heated at 90°C for 30 minutes. The supernatant was used as template for the species specific PCR or stored at -20°C for future use.

2.2.3 Positive and Negative Controls

Positive controls for *Theileria parva* were liquid genomic DNA provided by Joseph Mubanga while Trypanosomiasis positive controls were liquid genomic DNA provided by Heba Ahmed and Louise Hamill**,** all PhD students at the University of Edinburgh**.** The negative controls were obtained by punching clean FTA cards during the sample preparation stage for the day. The negative controls were then processed alongside the other samples from the washing stage to the DNA elution stage. At least one positive control and one negative control were included with each PCR reaction.

2.2.4 PCR oligonucleotide primers

PCR oligonucleotide primers were obtained from Integrated DNA Technologies (IDT), UK**.** The species specific oligonucleotide primers used for each pathogen will be outlined in the relevant species specific PCR reaction section.

2.2.5 PCR reagents

PCR reagents used in the laboratory were obtained from Bioline Ltd UK. BIOTAQ™ Red DNA Polymerase was used for the PCR reactions. BIOTAQ™ Red DNA Polymerase is purified from *Thermus aquaticus* and it contains a non-toxic and non-hazardous red dye. The red dye provides easy and quick

identification of reactions to which the enzyme has been added, and facilitates the confirmation of complete mixing. When the reaction is complete, a sample of the reaction mix can be loaded directly onto the agarose gel without the need for a loading buffer, since the mix is of a sufficiently high density to sink to the bottom of the well. The red dye migrates towards the positive electrode, thereby providing a means to monitor the progress of the electrophoresis. Other PCR reagents that were used in the reactions are 10x NH_4 reaction buffer (Bioline Ltd UK), 50 mM $MgCl_2$ solution (Bioline Ltd UK) and 100 mM dNTP Mix (Bioline Ltd UK). PCR reagents were aliquoted into 0.5 ml Eppendorf tubes and stored at -20°C until use. Each Eppendorf tube contained the volume of reagent needed for PCR reactions performed in a day. During PCR mastermix preparation, the PCR reagents were kept on ice.

2.2.6 Thermal cycling

Thermal cycling was conducted in a Peltier Thermal Cycler (MJ Research Inc, USA).

2.2.7 Agarose Gel Electrophoresis and visualisation of Amplicons

Agarose gel 1.5 % (w/v) electrophoresis was carried out after the PCR products were obtained. The agarose gel was prepared by adding 1.5 g of Agarose molecular grade (Bioline Ltd UK) to 100 ml of 1x concentrated Tris Borate EDTA Buffer (TBE buffer, 89 mM, Tris Borate, pH 8.3, containing 2 mM EDTA) (Sigma-Aldrich, Dorset, UK). This mixture was stained with Gel Red (Biotium, USA) at 4 μl per 100 ml and then allowed to set. PCR product samples were loaded into separate wells of the gel. Prior to each run, each gel contained positive and negative controls, molecular weight markers and PCR product samples.

Electrophoresis was conducted at 100 volts for between 40 minutes and one hour. Visualisation of results was done using the Gel Doc 2000 (BIORAD, USA). In gel electophoresis, DNA is loaded at the cathode and migrates towards the positively charged anode. Shorter DNA fragments migrate faster than longer DNA fragments.

2.2.8 DNA ladder

The DNA ladder used was the ExcactGene Low Range Plus DNA Ladder (Fisher Scientific International Inc., Canada). This was a 1 ml premixed ladder (0.5 μg/10 μl) in loading dye (10 mM EDTA, 10 % glycerol, 0.05 % bromophenol blue and 0.17 % SDS) and was ready to use. This 100 base pair (bp) ladder was run alongside PCR product samples on 1.5 % agarose gel during electrophoresis. A volume of 10 μl was used each time on the agarose gel during running of samples. The ladder was used to confirm the size of the PCR products being investigated.

2.2.9 Species specific polymerase chain reaction

The molecular diagnosis of *Theileria parva (T. parva)*, *Trypanosoma congolense (T. congolense)* (Savannah type), *Trypanosoma vivax (T. vivax)* and *Trypanosoma brucei (T. brucei)* was carried out at the University of Edinburgh by Polymerase Chain Reaction (PCR) using species specific primers.

The PCR reaction volume for each sample was 25 μl. The template DNA used in the 25 μl reaction mixture was 3 μl. For the positive controls, the amount of DNA template used in the 25 μl reaction mixture was 1 μl. The 25 μl PCR reaction mixture contained 10x NH_4 reaction buffer [160 mM $(NH_4)_2$ $S0_4$, 670 mM Tris-

HCl (PH 8.8 at 25°C), 0.1 % stabilizer] (BIOLINE LTD UK); 50 mM MgCl2 solution (final concentration 1.5 mM) (Bioline Ltd, UK); 800 μM total dNTP's (Bioline Ltd, London); 5U of BIOTAQ™ Red DNA Polymerase was used for the trypanosomiasis PCR while 1U was used for *T. parva* reaction. The *T. congolense* and *T. vivax* reactions each had 1 μM of each of the species specific forward and reverse primers while *T. parva* and *T. brucei* had 0.4 μM and 0.2 μM respectively. The reaction mixture volume was brought up to 25 μl using double distilled water.

2.2.9.1 *Theileria parva* PCR

Theileria parva species specific primers were used to amplify *T. parva* in the field samples (Table 2.2). The primers used were the forward IL3232 and the reverse IL4234 derived from the 104-kilodalton (p104) rhoptry antigen gene (Iams et al., 1990; Skilton et al., 2002). This set of primers can amplify a wide range of cattle-derived and buffalo-derived stocks of *T. parva* with no cross reactivity with other Theileria species such as *T. annulata, T. buffeli, T. lestoquardi, T. mutans* and *T. taurotragi*. The PCR reaction conditions used for *T. parva* amplification are outlined in Table 2.2. The amplicon generated by this set of primers is 276 bp in size.

2.2.9.2 Trypanosomiasis PCR

Table 2.2 shows the species specific primers for *T. congolense* (Savannah type), *T. vivax* and *T. brucei* that were used to amplify the pathogens in the field samples (Masiga et al., 1992; Moser et al., 1989; Skilton et al., 2002). The PCR reactions were carried out in 25 μl reaction volumes and were set up as explained earlier.

Table 2.2 outlines the PCR reaction conditions used for the amplifications as well as the size of the amplicons generated by the various sets of primers.

Table 2. 2 PCR primer sequences used in the 5′-3′ orientation and their reaction conditions

Parasite	*Primer*		*Amplicon (bp)*	**Reference**
T. parva	**P1**	GGCCAAGGTCTCCTTCAGAATACG		
	P2	TGGGTGTGTTTCCTCGTCATCTGC	276 bp	Skilton et al., 2002
T. parva PCR: 94 °C - 3 mins, 94°C - 45 secs x 35 cycles, 55°C - 45 secs, 72 °C - 1 min, 72°C - 5 mins.				
T. congolense (Savannah)	**P1**	GGACAAACAAATCCCGCACA		Masiga et.
	P2	CGAGAACGGGCACTTTGCGA	316 bp	al., 1992
T. congolense PCR: 94 °C - 3 mins, 94°C – 1 min x 35 cycles, 55°C – 2 mins, 72 °C - 2 min, 72°C - 5 mins.				
T. vivax	**P1**	CAGCTCGGCGAAGGCCACTTGCTGGGGTG		
	P2	TCGCTACCACAGTCGCAATCGTCGTCTCAAGG	400 bp	Masake et al. 1997
T. vivax PCR: 94 °C - 3 mins, 60°C – 1 min x 30 cycles, 55°C – 2 mins, 72 °C - 2 min, 72°C - 5 mins.				
T. brucei	**P1**	AGAACCATTTATTAGCTTTGTGC	177 bp	Moser et al. 1989
	P2	CGAATGAATAAACAATGCGCAGT		
T. brucei PCR: 94 °C - 3 mins, 94°C - 30 secs x 30 cycles, 60°C - 30 secs, 72 °C – 30 secs, 72°C - 5 mins.				

CHAPTER 3

Cattle Management Practices in Petauke District Zambia

3.1 Introduction

This chapter looks at cattle ownership, cattle management practices and farmers' perceptions of problems associated with owning livestock in Kasero and Makale veterinary camps of Petauke district in Eastern Province of Zambia. Assessment of these demographic and livestock management characteristics was determined by the use of a structured questionnaire.

3.1.1 Geography of Zambia

Zambia is a former British colony. It gained its independence on the 24th October, 1964. Zambia is located in the Central Plateau of Africa covering an area of about 752,614 square kilometres. It lies between latitudes 8^{o} and 18^{o} South and longitudes 20^{o} and 35^{o} East. The country shares borders with Tanzania and Democratic Republic of Congo to the North, Angola to the West, Namibia and Botswana to the South-west, Zimbabwe to the South, Mozambique to the South-east and Malawi to the East. Administratively, the country is divided into nine provinces, namely Central, Copperbelt, Eastern, Luapula, Lusaka, Northern, North-western, Southern and Western provinces (Figure 3. 1). These provinces are further divided into a total of seventy-three (73) districts. Lusaka is the capital city of Zambia and seat of Government. The Government comprises the Central and Local Authorities.

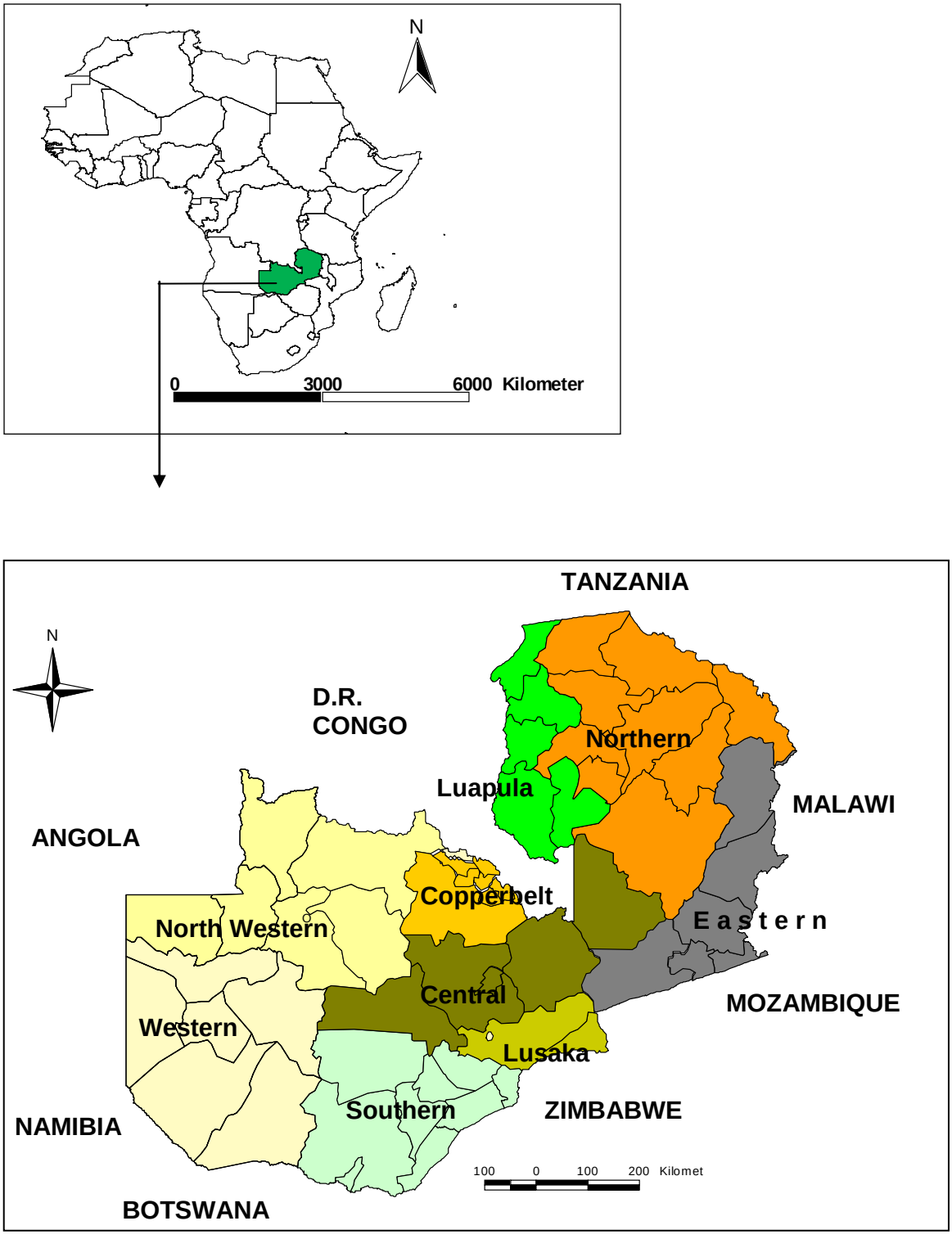

Figure 3. 1 Map of Zambia showing the nine provinces and eight neighbouring countries

3.1.2 Population engaged in agriculture

The population of the country is currently estimated at 12.4 million people (Central Statistical Office, 2000). Cattle are the most important type of livestock in Zambia. The bulk of livestock are kept in the traditional sector (about 80%). The livestock sector comprises 2,300,00 cattle, 97,600 sheep, 1,000,000 goats, 286,000 pigs, 2,500 donkeys, 850 horses, and 1,400,000 dogs (DVLD, 2004). Poultry production is estimated at 18 million broiler birds and 5 million layers. Twelve million birds are in the traditional sector (DVLD, 2004). Despite its importance, livestock ownership and livestock production is jeopardised by a range of animal diseases resulting in increased mortality rates and directly or indirectly affecting production (Van den Bossche and De Deken, 2002; Van den Bossche et al., 2005).

Some 75 per cent of Zambia's population is engaged in agriculture, largely subsistence farming (Central Statistical Office, 2000). There were a total of 1,300,000 agricultural households in Zambia as of October 2000 with 19.3 % being female headed. Eastern Province accounted for 17.7 per cent of these. Most (81.8 per cent) of the population in agricultural households was based in rural areas of Zambia (Central Statistical Office, 2000). Of the population in agricultural households countrywide, 47.4 per cent had only attained a primary level of education, and 35.8 per cent had not attained any level of education. The proportion of males and females who attained primary education was almost equal, 47.9 per cent and 46.9 per cent, respectively (Central Statistical Office, 2000). A total of 454,000 households raised livestock; with the number of goat-raising households constituting 38.7 per cent while cattle raising households making up 35.2 per cent (Central Statistical Office, 2000).

3.1.3 Government veterinary services in Zambia

For veterinary administration purposes, the Zambian Department of Veterinary and Livestock Development (DVLD) has its headquarters in Lusaka, the capital city of Zambia. Each of the country's nine provinces is headed by a Senior Veterinary Officer. The provinces are further divided into districts headed by Veterinary Officers. The districts are subdivided into veterinary camps that are manned by Veterinary Assistants.

3.2 Materials and Methods

3.2.1 Study site

3.2.1.1 Eastern Province:

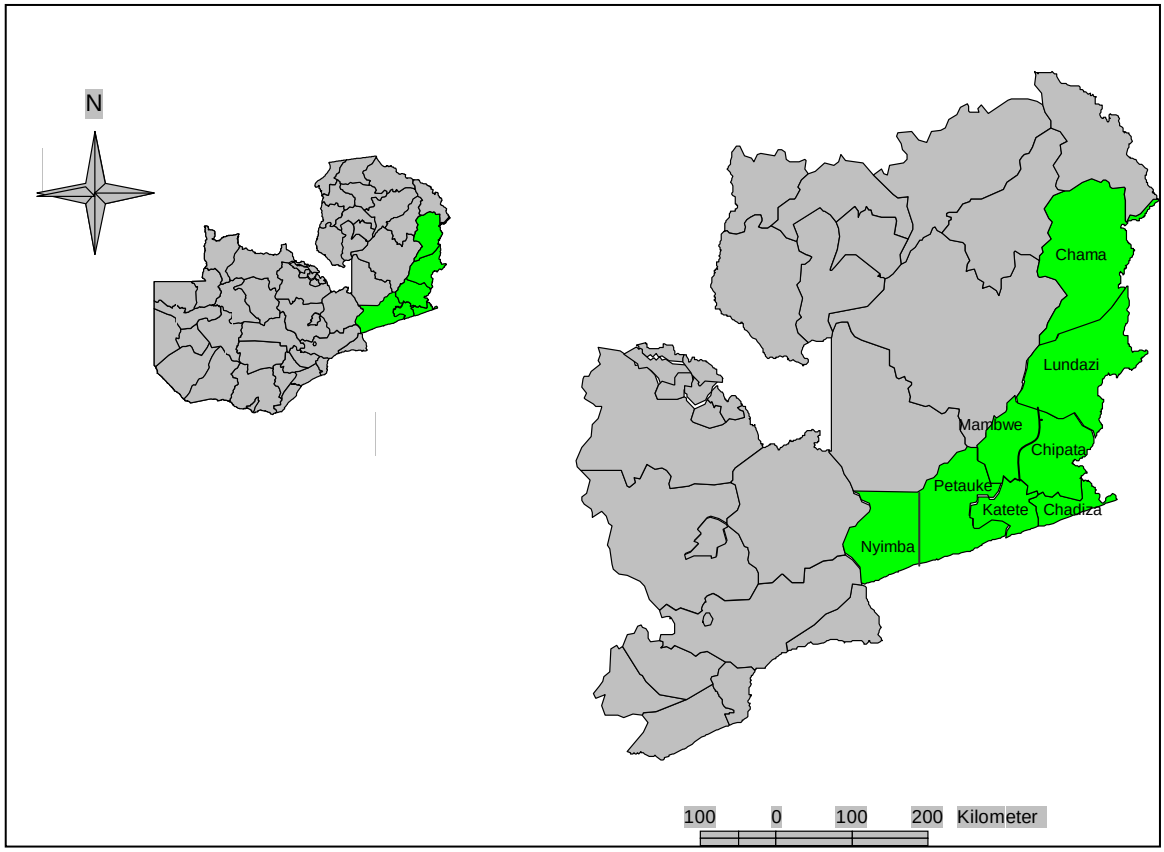

Figure 3. 2 Map of Eastern Province showing the eight districts including Petauke District

The Eastern Province of Zambia has a total surface area of approximately 69,106 km^2, about 9% of Zambia's total territory. It is divided into eight districts. These are Chipata, Chama, Lundazi, Chadiza, Mambwe, Nyimba, Katete and Petauke. It has a human population of 1,500,000 (Central Statistical Office, 2000) and lies between latitude 10° and 15° south and longitude 30° and 33° east. It is bordered by Malawi to the north-east and Mozambique to the south-east. The provincial capital is Chipata and the district that was selected for the study is Petauke. Topographically, the province is composed of two distinct zones: the plateau rising to 900-1200 m above sea level and the Luangwa valley at 300-600 m above sea level. The latter is drained by the Luangwa River. Like the rest of Zambia, the province experiences three distinct seasons: the warm rainy season (from November to April), the cool dry season (May to August) and the hot dry season (September to October) (Van den Bossche and De Deken, 2002). This area therefore has six months (May to October) of dry weather with no rain. The annual rainfall ranges from 800 -1000 mm, with most of the rains occurring between December and March.

3.2.1.2 Makale and Kasero Veterinary Camps, Petauke District

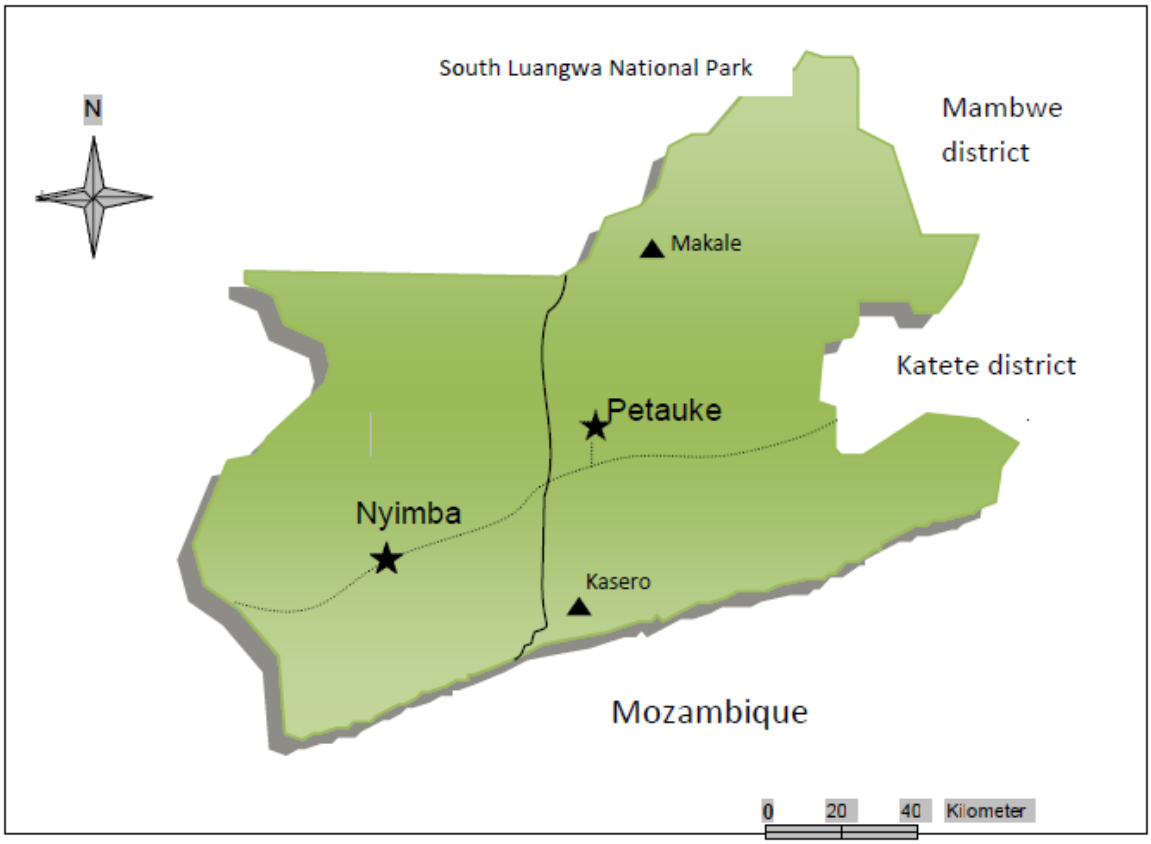

Figure 3. 3 Map of Petauke district showing the study sites of Kasero and Makale

Makale and Kasero were the two veterinary camps in Petauke District that were selected as areas for conducting the studies (Figure 3. 3). Petauke district is bordered by Nyimba district to the west, Serenje district of Northern Province in the north-west, Mpika district of Northern Province in the north, Mambwe district in the north-east and Katete district in the east. In the south, Petauke district borders Mozambique. Kasero veterinary camp lies more in the plateau area of Petauke district while Makale is located in the valley area near the Luangwa national park. The two areas are approximately 100 km apart.

3.2.2 Petauke District livestock populations

The Livestock populations in Petauke district are shown in Table 3. 1. Petauke district has a total number of 59,700 cattle, 36,000 goats and 58,600 pigs (DVLD, 2006). Cattle are the most important livestock species in the district followed by pigs and goats. Other livestock include sheep (200), donkeys (140) and rabbits (460). Poultry is dominated by chickens (132,800), followed by pigeons (7,900). Other poultry include guinea fowls (4, 100) and ducks (5,300) (DVLD, 2006).

Table 3. 1 Livestock populations in Petauke district (Source: DVLD Petauke district annual report, 2006)

Animal Type	*Population*
Cattle	59,794
Goats	36,029
Pigs	58,608
Sheep	211
Donkeys	140
Rabits	460
Guinea Fowls	4,176
Chickens	132,825
Ducks	5,302
Pigeons	7,942

3.2.3 Farming practices of people of Petauke District

Agriculture and trading are the major traditional economic activities of the people of eastern province. Most of the population practices mixed farming, combining both traditional pastoral and arable activities (Figure 3. 4). Most farmers concentrate on cattle keeping and other small stock mainly pigs and goats while also concentrating on growing maize, groundnuts and cotton (personal observation).

Figure 3. 4 A boy herding cattle in Petauke District, Zambia

3.2.4 Cattle management questionnaire

A structured questionnaire was used to investigate the demographic, cattle population and livestock management aspects in Kasero and Makale veterinary camps. The questionnaire was administered in February and March of 2008. Local enumerators who administered the questionnaire were initially trained in a classroom setting and had all their questions concerning the questionnaire

addressed. The second part of the training was in the field, where they administered the questionnaire together with the researcher (author). For purposes of the study, the following three definitions were used based on the Zambian 2000 Census report (Central Statistical Office, 2000).

Household: A household is a group of persons who normally live and eat together. These people may or may not be related by blood, but make common provision for food or other essentials for living and they have only one person whom they all regard as the head of the household. A household may also consist of one member.

Head of Household: This is the person who is considered to be the head by the other members of the household. He/She is the one who normally makes day-to-day decisions governing the running of the household. In a matrimonial household, the husband is usually taken as the head.

Livestock: This includes all cattle, pigs, goats, sheep and donkeys.

The questions and answers of the questionnaire were written down in English but farmers were asked and answered in the local language Chi Nyanja. The questionnaire consisted of two parts A and B. Part A contained identification information such as name of veterinary camp and date. Part B was further divided into three parts. The first part was for demographic data about the farmer and his family. The second part was about the farmers' livestock inventory while the third part was about cattle management practised by the farmers.

3.2.5 Selection of farmers for questionnaire administration

Farmer selection for questionnaire interview was based on the 2006/7 baseline and the 2008 treatment intervention studies. Only farmers that participated in the two studies were selected for interviews. This meant that only households with at least one cow could participate in the interviews. The interviews were conducted on a one to one basis and in such a manner as to have only one person to represent the household.

3.3 Results

3.3.1 Demographic

The demographic results of this chapter are those from cattle owning farmers in Kasero and Makale veterinary camps. The demographic assessment was carried out in order to determine certain characteristics of the farmers in the area such as their age, level of education and number of people in households. These characteristics would aid in understanding the type of livestock management in the area and its possible effect on cattle movement.

3.3.1.1 Number of male and female headed households

A total of 80 households were sampled in Kasero while 41 households were sampled in Makale (Table 3. 2). In Kasero, 67 households (84%) were headed by men while 13 households (16%) were female headed. Makale had 34 (83%) households headed by males while females headed 7 (17%) of households. For both areas, households headed by men were 101 (83.5%) while female headed households were 20 (16.5%). The number of households headed by men in Makale and Kasero was not significantly higher than the number headed by women ($x^2 = 0.013$, d.f. = 1, p = 0.91).

Table 3. 2 Number of male and female headed households in Kasero and Makale

Area	*Number of Male headed HH*	*Number of Female headed HH*	*Total number of HH*
Kasero	67 (84%)	13 (16%)	80
Makale	34 (83%)	7 (17%)	41
Both Areas	101 (83.5%)	20 (16.5%)	121

HH = Household

3.3.1.2 Household age distribution of questionnaire respondents

The average age of household heads in Kasero was 41.7 years (Table 3.3). The oldest household head was 81 years old while the youngest was 17 years old. Both the youngest and oldest household heads in Kasero were female. Five of the respondents did not have their age data included in the analysis because they either did not know their age or were not household heads.

Table 3. 3 Age of male and female household heads in Kasero

Variable	*N*	*N**	*Mean*	*Median*	*StDev*	*Min*	*Max*
Male	11	2	43.4	44.0	8.8	23	53
Female	64	3	41.4	39.5	14.5	17	81
Total	**75**	**5**	**41.7**	**42.0**	**13.8**	**17**	**81**

N = Missing data*

The oldest household head in Makale was 75 years old while the youngest was 24 years old (Table 3. 4). The oldest household head in Makale was a woman while the youngest was a man. The mean age of Makale household heads was 41.3 years. Two of the respondents did not have their age data included in the analysis because they either did not know their age or were not household heads.

Table 3. 4 Age of male and female household heads in Makale

Variable	*N*	*N**	*Mean*	*Median*	*StDev*	*Min*	*Max*
Male	33	1	40.6	40.0	9.17	24	59
Female	6	1	45.2	42.0	15.7	32	75
Total	**39**	**2**	**41.3**	**40.0**	**10.3**	**24**	**75**

N* = Missing data

3.3.1.3 Number of people in households and their age composition

From a total of 80 households interviewed, the mean number of people per household in Kasero was 7.6 (Table 3. 5). The minimum number of people per household was 1 while the maximum was 16. Makale had 41 households interviewed with the mean number of people per household of 6.8 (Table 3. 5). Makale had a minimum number of people of 2 per household and a maximum of 13.

Table 3. 5 Number of people in households by age composition in Kasero

Variable	*N*	*Mean*	*Median*	*StDev*	*Min*	*Max*
Kasero	**80**	**7.6**	**7.0**	**3.3**	**1**	**16**
Makale	41	**6.8**	**6.0**	**2.9**	**2**	**13**

3.3.1.4 Age distribution of school going children and herd boys

In Kasero and Makale veterinary camps, 43% of children under 18 years old from households that took part in the questionnaire study went to school (Table 3. 6). In Kasero alone, 43.3% of children under 18 years old went to school while in Makale the figure was 41.4%.

For children aged 12 years or below, 41.6% went to school in both Kasero and Makale. In Kasero alone, this figure was 43.5% while in Makale it was 38.4%. For children aged between 13 and 18 years, 44.9% were school going for both Kasero and Makale. In the same category, 42.7% were school going in Kasero and 51.1% were school going in Makale (Table 3. 6).

Table 3. 6 Percentage of school going children in study site

Area	*<= 12 years*	*13 – 18 years*	*< 18 years*
Kasero	43.5%	42.7%	43.3%
Makale	38.4%	51.1%	41.4%
Both areas	**41.6%**	**44.9%**	**43%**

A total of 117 (97.5%) of respondents gave the ages of their cattle herd boys (Table 3. 7). Four of the respondents did not give the herd boys' ages because they did not know. For both areas, the mean age of a herd boy was 11.2 years. Kasero had a mean herd boy age of 11 while Makale's was 11.6. The maximum age of a herd boy was 60 years while the minimum was 4 years old. Both the minimum and maximum ages for herd boys were in Makale.

Table 3. 7 Cattle herd boy age distribution in study site

Area	*N*	*Mean*	*Median*	*StDev*	*Min*	*Max*
Kasero	77	11.0	11.0	3.7	5	28
Makale	44	11.6	10.0	8.5	4	60
Both areas	**117**	**11.2**	**11.0**	**5.8**	**4**	**60**

3.3.1.5 Households raising cattle and other livestock

The questionnaire established that households raised cattle in addition to other livestock (Table 3. 8). Cattle were raised alone or in different combinations with pigs and goats. No farmer reported raising any sheep. As the study focused on farmers owning at least one cow, no farmers that had only other types of livestock without cattle were included in the study. Out of a total of 80 households in Kasero, 18.8% (15/80) raised cattle only, 27.5% (22/80) raised cattle and pigs, 6.2% (5/80) raised cattle and goats, and 47.5% (38/80) raised cattle, pigs

and goats. There were 13 female headed households in Kasero. Female headed households that raised cattle only, 30.8% (4/13), was the same as those households that raised cattle, pigs and goats. Female headed households that raised cattle and pigs accounted for 38.4% (5/13). There were no female headed households that raised cattle and goats. There were a total of 67 male headed households in Kasero. Slightly over half, 50.7% (34/67), raised cattle, pigs and goats. Cattle and goats raising households accounted for 7.5% (5/67) while cattle and pigs raising households accounted for 25.4% (17/67) of the total. There were only 16.4% (11/67) of male headed households that raised only cattle.

Makale had a total of 41 households that raised cattle in addition to the different types of livestock already mentioned (Table 3. 8). Cattle only raising households accounted for 7.3% (3/41), while households raising cattle and pigs were 22% (9/41). Cattle and goats raising households and cattle, pigs and goats raising households accounted for 24.4% (10/41) and 46.3% (19/41) respectively. There were only 7 female headed households in Makale. Households raising cattle and pigs, cattle and goats and cattle, pigs and goats households all accounted for 28.6% (2/7) each. The percentage of female headed households raising only cattle accounted for 14.2% (1/7). The male headed households in Makale were 34. Half of these households raised cattle, pigs and goats. Households that raised cattle and goats accounted for 23.5% (8/34) while those that raised cattle and pigs accounted for 20.6% (7/34). Cattle only raising households accounted for 5.9% (2/34). The interpretations and relationships of household demographics for both Kasero and Makale are given in the discussion at the end of this chapter.

Table 3. 8 Households raising cattle and other livestock in Kasero and Makale

Area	*Sex of Household Head*	*Cattle only*	*Cattle and Pigs*	*Cattle and Goats*	*Cattle Pigs Goats*	*Total*
Kasero	**Male**	11 (16.4%)	17 (25.4%)	5 (7.5%)	34 (50.7%)	67
	Female	4 (30.8%)	5 (38.4%)	0	4 (30.8%)	13
	Total	15 (18.8%)	22 (27.5%)	5 (6.2%)	38 (47.5%)	80
Makale	**Male**	2 (5.9%)	7 (20.6%)	8 (23.5%)	17 (50.0%)	34
	Female	1 (14.2%)	2 (28.6%)	2 (28.6%)	2 (28.6%)	7
	Total	3 (7.3%)	9 (22.0%)	10 (24.4%)	19 (46.3%)	41

3.3.1.6 Percentage of church going people in study site

The mean percentage of people attending church for both Kasero and Makale was 80% (713/891) (

Table 3. 9). Kasero's mean percentage for people attending church was 77% (471/610) while for Makale it was 86% (242/281).

Table 3. 9 Percentage of church going people in study site

Area	*People in HH*	*People who attend church in HH*	*Percentage attending church in HH*
Kasero	610	471	77%
Makale	281	242	86%
Both areas	891	713	80%

3.3.2 Livestock Inventory

Livestock inventory was carried out in order to find out the numbers, sex and types of cattle present in the two study sites of Makale and Kasero. This information would be useful in understanding how diseases present in the area might affect the animals. It has been shown that animals with different ages will be affected by diseases in different ways. The prevalence of T. congolense is highest in older animals while mortalities are highest in younger animals infected with T. parva (Mubanga, 2009).

3.3.2.1 Cattle composition in study site

Farmers were asked to classify their cattle into three categories. These were adult males, adult females and calves. Calves were defined as the young animals that were still suckling. Kasero respondents reported they had a total of 753 cattle while Makale respondents reported 220 cattle. Both areas had a total of 973 cattle (Table 3. 10). In Kasero, adult female cattle (41.04%) made up the largest category with adult male and calves categories accounting for 34.53% and 24.43% respectively. Adult male cattle in Makale made up the largest category (45.9%) followed by adult female (39.1%) and calves (15%). Overall, adult female cattle from both areas accounted for 40.6% while adult male cattle accounted for 37.1% of all animals in the areas. Calves from both areas accounted for 22.3% of all animals. There were more adult female cows than any other cattle in the other 2 categories (Table 3. 10). This was statistically significant ($x2 = 12.9$, d.f. = 2, $p < 0.05$).

Table 3. 10 Cattle compositions in study site

Area	*Adult male cattle*	*Adult female cattle*	*Calves*	*Total cattle*
Kasero	260 (34.53%)	309 (41.04%)	184 (24.43%)	753
Makale	101 (45.9%)	86 (39.1%)	33 (15.0%)	220
Both areas	361 (37.1%)	395 (40.6%)	217 (22.3%)	973

3.3.2.2 Average household herd sizes, compositions and total cattle

The average household herd sizes, compositions and total cattle are shown in Table 3. 11. The mean number of all cattle per household for the two areas was 8. For both Kasero and Makale, the mean number of adult male cattle per household was 3 while for adult female cattle and calves was 3.3 and 1.8 respectively. In Kasero, the mean number of adult male cattle per household was 3.2 while for adult female cattle and calves was 3.9 and 2.3 respectively. The mean number of adult male cattle per household was 2.5 in Makale. For adult female cattle and calves the mean number per household was 3.9 and 2.3 respectively. The mean number of cattle per household in Kasero was 9.4 while it was 5.4 in Makale.

Table 3. 11 Average household herd sizes, compositions and total cattle

Area	*Adult male cattle per household*	*Adult female cattle per household*	*Calves per household*	*Average cattle per household*	*Number of cattle owners*	*Total cattle*
Kasero	3.2	3.9	2.3	9.4	80	753
Makale	2.5	2.1	0.8	5.4	41	220
Both areas	3.0	3.3	1.8	8.0	121	973

3.3.3 Cattle Management

3.3.3.1 Important cattle diseases as ranked by farmers

Farmers ranked cattle diseases in order of importance during the questionnaire study. Each farmer could name up to three diseases (starting with the most important) that he/she thought was an important disease constraint (Table 3. 12). The cattle disease constraint that was named first was only considered for the first ranking. In cases where second and third disease constraints were provided, they were only considered for second and third ranks respectively. Farmers had widely used local names for two of the most common diseases in the study area. The local veterinary personnel were in agreement about the local names corresponding with the English names of the diseases based on their experience of examining sick animals in the area.

Table 3. 12 Importance rank of cattle diseases in Kasero (percentage of respondents)

	Rank 1 (%)	Rank 2 (%)	Rank 3 (%)
Trypanosomiasis	2.5	20.4	12
ECF	**85**	6.1	2
Other TBD	2.5	8.2	6
Helminths	0	0	0
Footrot	2.5	**30.6**	16
Lumpy skin disease	2.5	28.6	24
Other diseases/conditions	1.25	6.1	**40**
Don't know	3.75	0	0

Key: Rank 1 = most important disease, Rank 3 = third most important disease

TBD = tick borne diseases, 3 Highest ranked percentages in each rank are bold and underlined

Farmers were given the opportunity to name up to three cattle diseases that they considered important in the area. Some farmers were able to name and rank

three diseases that they considered important, others named two while others could only manage to name one or no disease at all. The cattle diseases that farmers named as being important are listed in Table 3. 12 and Table 3. 13. For Kasero, farmers ranked ECF (85%) as the most important disease in the area. Those farmers that named a second disease ranked footrot (30.6%) as topmost while other diseases or conditions (40%) were ranked third. Other diseases or conditions included eye infections, diarrhoea and topical wounds. In Makale, 90.2% farmers ranked trypanosomiasis as the most important disease in the area. Footrot was considered as the next important disease in the area (46.1%). The diseases that were named for third rank position in Makale were the other tick-borne diseases (23.1%).

Table 3. 13 Importance rank of cattle diseases in Makale (percentage of respondents)

	Rank 1 (%)	Rank 2 (%)	Rank 3 (%)
Trypanosomiasis	**90.2**	0	0
ECF	0	7.7	0
Other TBD	0	**23.1**	0
Helminths	0	7.7	0
Footrot	0	**46.1**	0
Lumpy skin disease	0	0	0
Other diseases/conditions	0	15.4	0
Don't know	9.8	0	0

Key: Rank 1 = most important disease, Rank 3 = third most important disease
TBD = tick borne diseases, 3 Highest ranked percentages are bold and underlined

3.3.3.2 Important cattle production constraints as ranked by farmers

Farmers named what they considered to be important cattle production constraints in their area. Each farmer had the opportunity to name up to three constraints. The cattle production constraint that was named first was only considered for the first ranking. In cases where second and third production

constraints were provided, they were only considered for second and third ranks respectively. The cattle production constraint named by farmers were cattle diseases, lack of water, grazing land shortage, stock thefts, wild predators and inadequate veterinary services. Constraints labelled as "other" included problems such as poor rains and lack of finances. Cattle diseases (83.8%) were described as the top ranking cattle production constraint by Kasero farmers (Table 3. 14). The second ranked problem was lack of water (50%) followed by grazing land shortage (42.1%).

Table 3. 14 Importance ranking of main cattle production constraints in Kasero (percentage of respondents)

Constraint	*Rank 1 (%)*	*Rank 2 (%)*	*Rank 3 (%)*
Cattle diseases	**83.8**	21.4	0
Lack of water	2.5	**50**	15.8
Grazing land shortage	3.7	14.3	**42.1**
Stock theft	0	1.8	10.5
Wild predators	0	0	0
Inadequate vet services	3.7	1.8	31.6
Other	6.3	10.7	0

Key: Rank 1 = most important constraint, Rank 3 = third most important constraint
3 Highest ranked percentages are bold and underlined

In Makale, 90.2% of respondents considered cattle diseases as important cattle production constraints (Table 3. 15). The second most important cattle production constraint in Makale was the grazing land shortage (54.5%). The third ranked cattle production constraint was the category "other" which like in Kasero included problems such as poor rains and lack of finances.

Table 3. 15 Importance ranking of main cattle production constraints in Makale (percentage of respondents)

Constraint	*Rank 1 (%)*	*Rank 2 (%)*	*Rank 3 (%)*
Cattle diseases	**90.2**	0	
Lack of water	0	0	0
Grazing land shortage	2.5	**54.5**	0
Stock theft	0	4.6	0
Wild predators	0	27.3	0
Inadequate vet services	0	13.6	0
Other	7.3	0	**100**

Key: Rank 1 = most important constraint, Rank 3 = third most important constraint
3 Highest ranked percentages are bold and underlined

3.3.3.3 Farmers' perceptions on reducing cattle diseases

Farmers listed ways in which they thought cattle diseases would be best reduced. The solutions which farmers provided for reducing cattle diseases in their areas are listed below (Table 3. 16). The most popular solution for farmers (32.5%) in Kasero was lowering the cost and improving the availability of acaricides. This was followed by 30% of farmers who suggested that farmer training on all aspects of cattle production would help lower diseases. A total of 23.8% of Kasero farmers felt that lowering the cost and improving the availability of veterinary drugs would greatly help reduce cattle diseases. Other farmers in Kasero suggested having better veterinary services (18.8%) and building diptanks (10%). The "other" category had suggestions that veterinary drugs should be sourced from reliable sources. A total of 6.2% of farmers in Kasero were not sure how cattle diseases could be reduced in their area.

Table 3. 16 Farmers' perception on reducing cattle diseases in study site (percentage of respondents)

Intervention	*Kasero Veterinary Camp*	*Makale Veterinary Camp*
Better veterinary services	18.8%	**14.6%**
Farmer training	**30%**	9.8%
Lower cost and improved availability of vet. drugs	**23.8%**	**26.8%**
Lower cost and improved availability of acaricides	**32.5%**	4.9%
Build diptanks	10%	0
Deploy targets for tsetse control	0	**39%**
Helminths control	0	2.4%
Other	1.2%	0
Not sure	6.2%	7.3%

3 highest ranked percentages in each area are bold and underlined

In Makale, the suggestions made by farmers on reducing cattle diseases in their area were deploying targets for tsetse control (39%), lowering the cost and improving availability of veterinary drugs (26.8%) and having better veterinary services (14.6%). Other suggestions from Makale farmers, were farmer training (9.8%), lowering the cost, availability of acaricides (4.9%) and controlling helminths (2.4%). A total of 7.3% of farmers in Makale were not sure how cattle diseases could be reduced in their area. The differences in terms of responses from the farmers between areas and their related problems are presented in the discussion at the end of this chapter.

3.3.3.4 Farmers applying acaricide in the study site

In Kasero, 92.5% (74/80) of questionnaire respondents said they applied acaricide onto their animals during the year (Table 3. 17). In Makale, 48.8% (20/41) of farmers said they applied acaricide onto their animals (Table 3. 17). Overall, application of acaricide depended on whether ticks could be seen on the animals and whether money was available for buying the acaricide.

Table 3. 17 Percentage of farmers applying acaricide on cattle

Area	*Farmers applying acaricide on cattle*
Kasero	74/80 (92.5%)
Makale	20/41 (48.8%)

3.3.3.5 Number of cattle dying one year prior to questionnaire administration

Farmers provided data on the number of their animals that died during 2007 (Table 3. 18). This was during one year prior to the administration of the questionnaire. In Kasero, the total number of animals that were reported dead by farmers was 120 while in Makale it was 16. The suspected causes of death as suspected by the farmers in Kasero were ECF (12.7%), abortion (0.13%) and unknown causes (3.05%). For Makale, the suspected causes of death were trypanosomiasis (4.5%), snakebite (0.55%) and unknown causes (2.3%).

Table 3. 18 Number of cattle that died during 1 year prior to questionnaire interviews and causes of death (As suspected by farmers)

Area	*Suspected cause of death*	*Number of animals dead*	*Number of animals reported*	*Mortality rate as reported by the farmers (%)*
Kasero	Abortion	1	753	0.13
	ECF	96		12.7
	Unknown	23		3.05
Makale	Trypanosomiasis	10	220	4.5
	Snakebite	1		0.55
	Unknown	5		2.3

3.3.3.6 Source and administration of veterinary drugs in study area

Farmers provided data on where they source their veterinary drugs and who administers the drugs when the animals are sick. Most farmers in both Kasero (57.5%) and Makale (60%) get their veterinary drugs from the Petauke district veterinary office (Table 3. 19). Next as sources for veterinary drugs in Makale were the agricultural input and veterinary shops in Petauke and the neighbouring districts of Katete and Chipata (30%). A total of 10% of Makale farmers obtained their veterinary drugs from mobile traders. Kasero farmers get their veterinary drugs from other farmers (16.2%), mobile traders (13.8%) and agricultural input and veterinary shops (12.5%).

Table 3. 19 Source of veterinary drugs in the study areas

Area	*Source of veterinary drugs*	*Number of farmers*	*Percentage of Farmers (%)*
Makale	Veterinary office	24	60.0
	Shops	12	30.0
	Mobile traders	4	10.0
	Other farmers	0	
Kasero	Veterinary office	46	57.5
	Shops	10	12.5
	Mobile traders	11	13.8
	Other farmers	13	16.2

The people administering the drugs to the sick animals were listed by the questionnaire respondents as the following; the farmer himself, a farmer's relative, another farmer, a community livestock worker (CLW), or a veterinary official who could be a veterinary officer, livestock officer or a veterinary assistant. Kasero farmers responded that 72.5% treated their own animals (Table 3. 20). People who helped Kasero farmers treat their animals were other farmers (17.5%), farmer's relatives (6.25%) and veterinary officials (3.75%). In Makale, 68.85% of farmers treated their own cattle while other farmers helped in 17.1% of cases (Table 3. 20). Farmer's relatives and community livestock workers treated the animals 9.76% and 4.9% of the time.

Table 3. 20 People administering veterinary drugs in the study area

Area	*Person administering drugs*	*Number of farmers*	*Percentage of farmers (%)*
Kasero	Farmer	58	72.5
	Farmer's relative	5	6.25
	Another farmer	14	17.5
	CLW	0	0
	Veterinary official	3	3.75
Makale	Farmer	27	68.85
	Farmer's relative	4	9.76
	Another farmer	7	17.1
	CLW	2	4.9
	Veterinary official	0	0

3.3.3.7 People who first identify sick animals

Herd boys were identified as the people who most often identified first the sick animals in a herd for both Kasero (95%) and Makale (80.5%) (Table 3. 21). Farmers in Kasero identified sick animals first 5% of the times while in Makale they identified sick animals first 19.5% of the times (Table 3. 21).

Table 3. 21 Person who first notices sick animal in the study area

Area	*Person who first notices sick animal*	*Number of farmers*	*Percentage of farmers (%)*
Kasero	Herd boy	76	95
	Farmer	4	5
Makale	Herd boy	33	80.5
	Farmer	8	19.5

3.4 Discussion

The household demographics, livestock population and cattle management practices were studied in Kasero and Makale veterinary camps, Petauke District, Eastern province, Zambia. A total of 121 households were interviewed in the two camps. Farmers that participated in the study had 973 cattle of which 361 (37.1%) were adult male cattle, 395 (40.6%) were adult female cattle and 217 (22.3%) were calves.

3.4.1 Characteristics of household demographics

All the farmers in the study were traditional subsistence farmers owning cattle and other livestock and also practicing crop farming. The percentage of male headed households was greater than that for female headed households in both areas of the study. This was comparable to the provincial average which had 80.2% male headed households and 19.8% female headed households (Central Statistical Office, 2000). The average age of household heads in Kasero was 41.7 years while that for Makale household heads was 41.3 years. Kasero had a slightly higher mean number of people per household (7.6) than Makale (6.8).

Less than half the children below 18 years in Kasero and Makale went to school. For children between 13 and 18 years old, the percentage of children who went to school in Kasero was 42.7% while it was 51.1% in Makale. The average age of a herd boy who herded the cattle during the day was 11.2 years for both areas. The maximum age of a cattle herder was 60 while the minimum was 4 years old. The 60 year old herder was a farmer who preferred to herd his own animals. Most farmers employed a herd boy who looked after their cattle seven days a week. The majority of the herd boys did not go to school and were not related to the

farmer. This is reflected in the fact that less than half the children in the area did not go to school. The farmer provides the herd boys accommodation and food and after a period of four to five years the herd boys are paid a female animal and this signifies the end of the contract term for herding. Only 18.8% of households raised cattle alone without any other livestock in Kasero while the number of Makale households raising only cattle was 7.3%. Overall, the number of households raising cattle, pigs and goats was higher than the number raising cattle and pigs or cattle and goats. Cattle, pigs and goats are the main livestock species found in this area (Simukoko et al., 2007). Having other types of livestock like pigs and goats enables the farmer to have a buffer in times of difficulties. He can sell off the cheaper animals first should he need urgent money before resorting to selling cattle which are more expensive.

The majority of people in the study area attended church at least once per week. Most people attended church on Sundays with a few attending on Saturdays and Wednesdays. Around 80% of people attended church in the study area. Because most farmers employed herd boys, attendance of church did not have an effect on how their cattle were managed on church-days. Only a few farmers acknowledged that on weekends they gave the herd boys time to rest or attend church.

3.4.2 Cattle compositions and average household herd sizes

The total number of people living in households that participated in the study was 973. Farmers in Kasero had a total of 753 cattle while Makale farmers had 220 cattle. Adult female cattle from both areas accounted for 40.6% of animals while adult male cattle accounted for 37.1%. Calves from both areas accounted

for 22.3% of the total number. The mean number of all cattle per household for the two areas was 8. The mean number of adult male cattle in the study area per household was 3 while for adult female cattle and calves it was 3.3 and 1.8 respectively.

3.4.3 Cattle management

Cattle management practices in Kasero and Makale are such that cattle are almost always in groups. These groups may comprise of cattle belonging to several people but being kraaled and herded together. Many households employ full time herders who can be boys as young as 4 years old. Cattle are normally herded separately from goats. Though each household has their own herder, the livestock of the village often meet and mix in the communal grazing areas nearby the villages. The grazing areas are natural pastures which are the cattle's main food supply. After the farmers harvest their crops from around May, cattle have access to crop residues of maize and groundnuts. When these residues run out there is a problem of grazing land and farmers have to move further from the villages to feed their animals. It is probable that in Makale Veterinary Camp, as herders search for grazing areas for their cattle, they move closer to the Kafue National Park which has abundant game that act as reservoirs for trypanosomes. They may therefore put their animals at greater risk of trypanosomiasis during this period.

During the rainy season from November to April cattle have drinking water from within the grazing areas in the form of puddles, ponds and nearby streams. In the dry season from May to October, Kasero farmers have a serious problem of finding drinking water for their animals as the streams run dry. Most of them

resort to getting water from wells for their animals or walking long distances. In Makale, streams throughout the dry season do have water. Cattle are let out of their kraals in the morning between 06:00 h and 08:00 h and return in the evening between 16:00 h and 18:00 h. During the rainy season cattle are always herded while in the dry season they are let out of their kraals and allowed to wander. They are collected by the herders before dark and put back in the kraals. Cattle are rarely left outside overnight because of the danger of stock theft. Farmers do not control the mating of their animals and calving occurs throughout the year.

3.4.4 Cattle diseases

Kasero farmers ranked ECF as the most important cattle disease followed by footrot and other diseases such as eye infections, diarrhoea and wounds. In Makale, farmers ranked trypanosomiasis as the most important disease and footrot was considered as the second most important disease. In Kasero, the total number of cattle that were reported dead by farmers was 120 while in Makale it was 16. The suspected causes of death as reported by the farmers in Kasero were ECF, abortion and unknown causes. For Makale, the suspected causes of death were trypanosomiasis, snakebite and unknown causes. Kasero farmers considered ECF as the most important disease while Makale farmers ranked trypanosomiasis as the most important. This ranking was not surprising as it has been shown by other researchers that trypanosomiasis is more prevalent in the northern villages of Petauke (where Makale is located) while ECF is in the southern areas where Kasero is located (Mubanga, 2009). This was in line with the general belief that the ECF is endemic in Kasero while trypanosomiasis is endemic in Makale.

All animals in the study area were grazed for an average of nine hours during the day and kept in kraals during the night. Kraaling is also practised in other southern African countries and is a long-established custom and is regarded as the right thing to do by many cattle owners as it is supposed to be a protective measure against wild predators (Reed et al., 1974). The kraals in the study area were in all instances made of logs forming a circular fence and were not roofed. The size of the kraal depended on the size of the herd. In most cases the farmer built his kraal in an area which would include an anthill to provide raised ground. This would provide an area of high dry ground for animals in the rainy season. It was found that dry ground provided by the anthills in the kraals was in most cases inadequate and lead to a lot of animals standing on lower ground which was poorly drained because of the dung and wet mud. The kraals where cattle are kept at night play an important role in the management of cattle. The cattle are kept in kraals for at least 12 hours and during the rainy season the ground becomes heavily contaminated with dung and mud making ideal conditions for transmission of disease pathogens (Kaufmann et al., 1993). This may be the reason that footrot was reported as an important disease in the area. It has been shown that frequent changing of the cattle holding site reduces the risk as measured by disease pathogen densities in the soil and increased the weight gain of the animals (Kaufmann et al., 1993). This practice of frequent changing of kraals during the rainy season was not observed in Petauke District during the study period.

3.4.5 Cattle production constraints

Cattle diseases were described as the top ranking cattle production constraint by both Kasero and Makale farmers. In Kasero the second ranked problem was lack of water followed by grazing land shortage. In Makale, the second most important cattle production constraint was grazing land shortage followed in third place by problems such as poor rains and lack of finances. In Botswana, the lack of water for cattle and overgrazing has been shown to seriously impair body condition and reproductive performance (Reed et al., 1974). Grazing land shortage was cited as a cattle production constraint in both Kasero and Makale. Extremes in the size of the grazing pasture has been shown to cause excessive walking and time spent walking decreases in correspondence with grass availability (Arave C. W., 1981).

3.4.6 Farmers' perceptions on reducing cattle diseases

ECF was seen as the most important disease in Kasero by the farmers. As a result, their ideas on reducing cattle diseases are targeted specifically at this disease. The majority of farmers in Kasero thought that lowering the cost and improving the availability of acaricides would greatly help reduce cattle diseases. Other farmers suggested that building diptanks for cattle dipping would greatly reduce cattle diseases. Other suggestions from farmers that were not directly related to ECF control included provision of farmer training, lowering the cost and improving the availability of veterinary drugs and having better veterinary services.

Like in Kasero, farmers in Makale made suggestions on reducing cattle diseases by focusing mainly on the most important disease in their area, trypanosomiasis.

Suggestions made by Makale farmers included deploying targets for tsetse control, lowering the cost and improving availability of veterinary drugs and having better veterinary services. Other suggestions were farmer training, lowering the cost and improving the availability of veterinary drugs and controlling helminths.

Because of the different types of diseases in the two areas, almost all people in Kasero said they applied acaricide onto their animals during the previous year. In Makale, less than half the farmers said they applied acaricide onto their animals. Overall, application of acaricide depended on whether ticks could be seen on the animals and whether money was available for buying the acaricide.

3.4.7 Source of veterinary drugs

Most farmers in both Kasero and Makale obtained their veterinary drugs from the Petauke district veterinary office. In Makale 30% of farmers sourced their veterinary drugs from agricultural input and veterinary shops in Petauke and the neighbouring districts of Katete and Chipata. A total of 10% of Makale farmers obtained their veterinary drugs from mobile traders. Kasero farmers obtained their veterinary drugs from other farmers, mobile traders and agricultural input and veterinary shops in Petauke, Katete and Chipata districts. The presence of mobile traders in the area is of some concern to the farmers as they think that some of the veterinary drugs supplied by them are of questionable quality (DVO, 2008).

3.4.8 Treatment of animals

Majority of Kasero and Makale farmers treated their own sick animals. Other people who helped Kasero farmers treat their animals were other farmers, farmer's relatives and veterinary officials. In Makale, those who helped farmers treat their cattle were other farmers, farmer's relatives and community livestock workers.

Herd boys were identified as the people who most often identified first the sick animals in a herd for both Kasero and Makale. A majority of farmers employed the herd boys to look after their animals seven days a week. The average age of herders for both areas was 11.2 years but they could be as young as 4 or 5 years old. Considering that herders are the first people to notice sick animals and that farmers in most cases buy drugs or report sick animals to the veterinary office when the disease is already advanced (DVO, 2008) this could be a result of these young herders not being able to notice sick animals in the early stages of illness. Furthermore, the herd boys are not included or invited to farmer training sessions organized by the veterinary department and livestock non-governmental organisations and yet are expected to first notice and tell the farmer when the animal is sick. A change in the target groups of extension messages from the veterinary department and NGO's to include herders may be warranted.

CHAPTER 4

Investigations of cattle movement behaviour using two-dimensional motion sensors in Petauke District, Zambia

4.1 Introduction

This chapter looks at whether two-dimensional motion sensors can be used to investigate the movement behaviour of traditionally managed cattle in sub-Saharan Africa. Icetag™ two-dimensional motion sensors (pedometers) were used to assess whether they could quantify the cattle movement behavioural characteristics of walking, standing and lying down in animals under an African traditional management system. The two-dimensional motion sensors were also used to assess whether any detected changes in cattle movement behaviour were associated with variations in the cattle's haemoglobin levels, a measure considered to be an objectively verifiable indicator for disease. Cattle with chronic forms of trypanosomiasis have been reported to be lethargic with low levels of haemoglobin (Naessens, 2006; Sekoni et al., 1990).

4.2 Materials and Methods

4.2.1 Study site identification

The study sites were in Kasero and Makale Veterinary Camps, Petauke District, Eastern Province of Zambia. Kasero lies more in the plateau area of Petauke district to the south while Makale is located northerly in the Luangwa Valley area near the Luangwa national park (see map in Chapter 3, Figure 3.3). The two veterinary camps were purposively selected from camps that had high prevalence of trypanosomiasis and theileriosis in previous surveys conducted in the area (Mubanga, 2009; Sinyangwe et al., 2004). The two areas were geographically well dispersed across Petauke District, being approximately one hundred kilometres apart and were also accessible throughout the year regardless of the season.

4.2.1.1 Geo-referencing of sampling sites

Sampling sites were geo-referenced using a handheld global positioning system (GPS) device, Etrex 12 channel GPS (Garmin, USA) as outlined in Chapter two (Section 2.1.6).

4.2.1.2 Recruitment of farmers to the study

One to two days before selection of cattle for the study at each village, local cattle owners were requested through the local district veterinary office to present their animals for examination at designated crushpens. This was in addition to the sensitization carried out at the beginning of the study (Chapter 2, section 2.1.1.). Cattle were presented for clinical examination and/or attachment of motion sensors at the designated crushpen by their owners or herdsmen.

4.2.2 Study design

4.2.2.1 Cattle selection and pairing

A total of 432 cattle were presented at the pre-selection stage in both Makale and Kasero veterinary camps. All animals presented by farmers had their haemoglobin levels measured using a Hemocue Hb 201+ haemoglobinometer (HemoCue AB, Ängelholm, Sweden). The haemoglobin values were measured and recorded for each animal as detailed in Chapter 2 (Section 2.1.4.1). All animals screened were identified by ear tags, name of the animal, owner's name and village.

A range of haemoglobin values were obtained from the pre-selection stage of the experiment and used to select 20 animals for the attachment of motion sensors at

each site. The selected 40 animals were then paired and later one motion sensor was attached to each hind leg of the animal. Each veterinary camp thus had ten pairs of cattle with motion sensors attached. The 40 animals were selected such that each pair included the most disparate haemoglobin values possible for pairs while still satisfying the following criteria:

- One animal in the pair had a comparatively low haemoglobin level in relation to the other animal
- All animals in the pairs were as close as possible the same age
- All animals in the pairs were the same sex
- All animals in the pairs were co-grazing and belonged to the same kraal
- Draft animals were excluded from the study

After the selection of the 10 co-grazing pairs at each site, the owners were informed about the next stage of the study and asked to present their animals for motion sensor attachment.

4.2.2.2 Motion sensor crossover validation design

A cross-over design was used to increase the chances of detecting an abnormally functioning motion sensor. This was achieved by exchanging the motion sensors in each co-grazing pair of animals on day seven of motion sensor attachment. The design enabled detection in the event of erroneous data such as from a faulty motion sensor unit.

4.2.2.3 Sample collection and duration of the study

Table 4. 1 Timeline of movement behaviour study

Day	***Procedure***
Day -2 (Pre-selection)	Cattle sampled for haemoglobin [Kasero (n = 211), Makale (n = 221)]. Haemoglobin data recorded.
0	Motion sensor attachment to twenty co-grazing cattle pairs. Haemoglobin data recorded.
7	Motion sensor data download. Motion sensor crossover exchange carried out within pairs. Haemoglobin data recorded.
14	Final motion sensor data download. Removal of motion sensors from animals. Haemoglobin data recorded.

Motion sensor attachment was carried out as detailed in Chapter 2 (Section 2.1.5). On the day of motion sensor attachment, haemoglobin values and other clinical parameters were measured and recorded (Chapter 2, section 2.1.3). This was repeated on days seven and fourteen of the experiment. On days seven and fourteen, motion sensor data was also downloaded to a laptop computer to avoid losing all data in the event of loss of or damage to a motion sensor unit. All animals in the co-grazing pairs therefore had four haemoglobin and clinical parameter readings (pre-selection, days 0, 7 and 14) in addition to the two weeks' worth of motion sensor data.

4.2.3 Data output and storage

Data recording and storage was carried out as explained in Chapter 2 (Section 2.1.7). Clinical data was recorded onto cattle data collection forms (Table 4. 2). Motion sensor data was downloaded from the motion sensors in the field onto a laptop computer via a USB cable using the IceTag™ Analyser software (Research Version 2.003) and saved as ".csv" files before being exported to Microsoft Excel (2003). Motion sensor data output comprised four variables. Three of the variables were in the form of a percentage that represented the time the animal spent doing a particular activity at a particular time of day. In any given hour, data could be estimated as the percentage of time the animal spent standing, being active or lying down. The last variable of motion sensor data output was that of the number of steps that the animal took during the period of interest. The IceTag™ Analyser software actually allows for data breakdown as per second, per minute, per hour, per day or per week. To analyse the data at minute intervals or lower would have resulted in very large datasets. Analysing the data at daily or weekly intervals would have resulted in loss of detail from the data. For informative and convenience reasons, motion sensor data was aggregated and examined as averages at hourly intervals throughout this study.

Table 4. 2 Cattle Data Collection Form

Date…….. District: Petauke **Vet camp:…….**

Village:……. GPS Coordinates:……..…..

Name of owner:…….

Name of VA/VO:…….…….

Tag No	Pedometer No	Name of animal	Sex	Age	Weight (Kg)	M M, N,P,PP	Haemoglobin (g/dl)	Body condition score F+,F,F- M+,M,M- L+,L,L-	Rectal Temp (ºC)	Blood collected on FTA card? (Yes/No)

MM=Mucous Membranes, N=Normal, P=Pale, PP=Very Pale, VA=Veterinary Assistant, VO=Veterinary Officer
Body condition scores are F [fat], M [medium] and L [lean] subdivided into three categories with scores from 1 to 9. F+ [9], F[8], F-[7]; M+[6], M[5], M-[4]; L+[3], L[2] and L-[1]

4.2.4 Motion sensor data analysis

Data obtained on haemoglobin values from the haemoglobinometers were entered and stored in Microsoft Excel (2003). Pedometer data was downloaded from the pedometers in the field onto a laptop computer via a USB cable using the IceTag Analyser software (Research Version 2.003) and saved as ".csv" files before being exported to Microsoft Excel (2003). Depending on the applications required, the software that was used for data analysis was Minitab version 14 (Minitab Inc.), Microsoft Excel (2003) and IceTag Analyser software (Research Version 2.003) and ArcView GIS 3.2 (Environmental Systems Research Institute, Inc.). Calculations of summary descriptive statistics were carried out using Minitab version 14 (Minitab Inc.). Microsoft Excel (2003) and Minitab version 14 (Minitab Inc.) were used to draw graphs. The comparison of mean haemoglobin concentration values was done using 2-sample and paired t-test with Minitab 14. The 2-sample Wilcoxon rank sum test was used to test the equality of two population medians.

In order to visualize the behaviour of cattle over a 24 hour period, cattle movement behaviour profiles were created. Cattle movement behaviour profiles created a summary of movement behaviour of the animals in the two veterinary camps. Boxplots were used to visualize the summary of movement behaviour profiles of cattle in Makale and Kasero. Boxplots were made for each of the variables under investigation for standing, walking and lying down behaviour using Minitab version 14 (Minitab Inc.).

The raw motion sensor data was converted to a data matrix for principal components analysis by making each animal's behaviour in 24 hours be defined by seventy two variables, each of which represented the mean percentage of time an animal spent standing, lying down or walking during a given hour over the period of the whole experiment (24 hours x 3 variables of behaviour = 72 variables). The data matrix was therefore, a rectangular block of data having columns and rows with each row corresponding to an individual animal and each column corresponding to a variable (percentage of time animal spent standing, walking or lying) at a certain hour over a twenty four hour period.

The overall aim of using the Principal Components analysis was to reduce the dimensionality of the 72 dimensional data set in order to simplify further analysis. The PCA was used to examine the data to try and detect any patterns present, identify the main sources of variability in the data and determine its effective dimensionality. The PCA was carried out using the statistical package Minitab version 14 (Minitab Inc.). Because the original variables measured different entities (i.e. hour of day and percentage of time spent by the animal doing a particular activity) the data was standardized before the analysis were conducted on the statistical package.

4.3 Results

The results in this chapter consist of the motion sensor data that were collected from the forty cattle on days seven and fourteen of the movement behaviour investigations at each of the two Veterinary Camps during 2006/07. Also presented are the haemoglobin results taken from these animals during the pre-selection stage and on days zero, seven and fourteen.

4.3.1 Haemoglobin values for cattle that had motion sensors attached in Petauke District

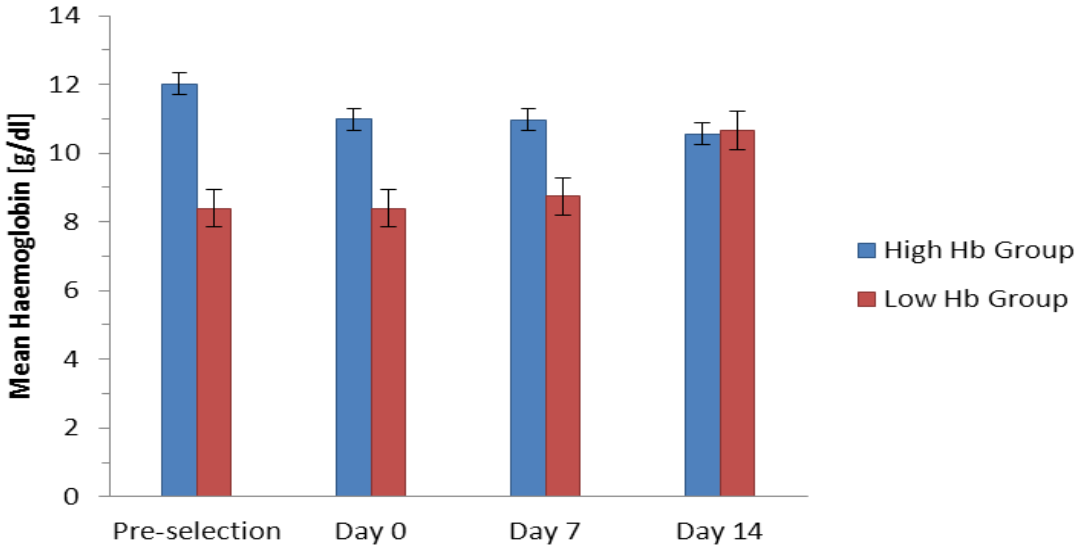

Figure 4. 1 Mean Haemoglobin (Hb) levels for cattle in the high and low haemoglobin groups that had motion sensors attached in Kasero Veterinary Camp. Error bars represent standard error for the data.

The mean haemoglobin values for cattle in the high and low haemoglobin groups during the pre-selection stage of the study and on days zero, seven and fourteen in Kasero Veterinary Camp are shown in Figure 4. 1. The same parameters are represented in Figure 4. 2 for Makale Veterinary camp. There was a significant difference between the mean haemoglobin values of the high and low groups in Kasero (paired t-test; mean difference = -1.78, 95% CI [-3.4, -0.2], $p < 0.05$).The

high haemoglobin group of animals had higher haemoglobin values than the low group. Makale animals had a highly significant difference between the mean haemoglobin values of the two groups with the high group having higher values (paired t-test; mean difference = 3.4, 95% CI [2.9, 3.9], $p < 0.001$).

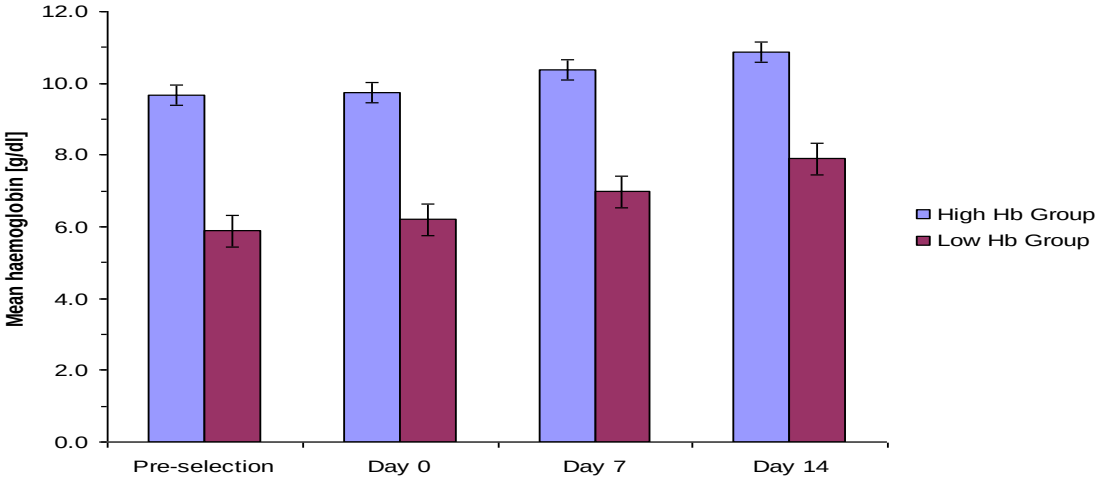

Figure 4. 2 Mean Haemoglobin levels for cattle in the high and low haemoglobin (Hb) groups that had motion sensors attached in Makale Veterinary Camp. Error bars represent standard error for the data

The mean haemoglobin values for cattle in Kasero and Makale during the pre-selection stage of the study and on days zero, seven and fourteen in Petauke District are shown in Figure 4. 3. The difference in mean haemoglobin values recorded for cattle screened in Kasero and Makale during the study was found to be significant (2-sample t-test; mean difference = 1.6, 95 % CI: [0.7, 2.4], $p < 0.01$).

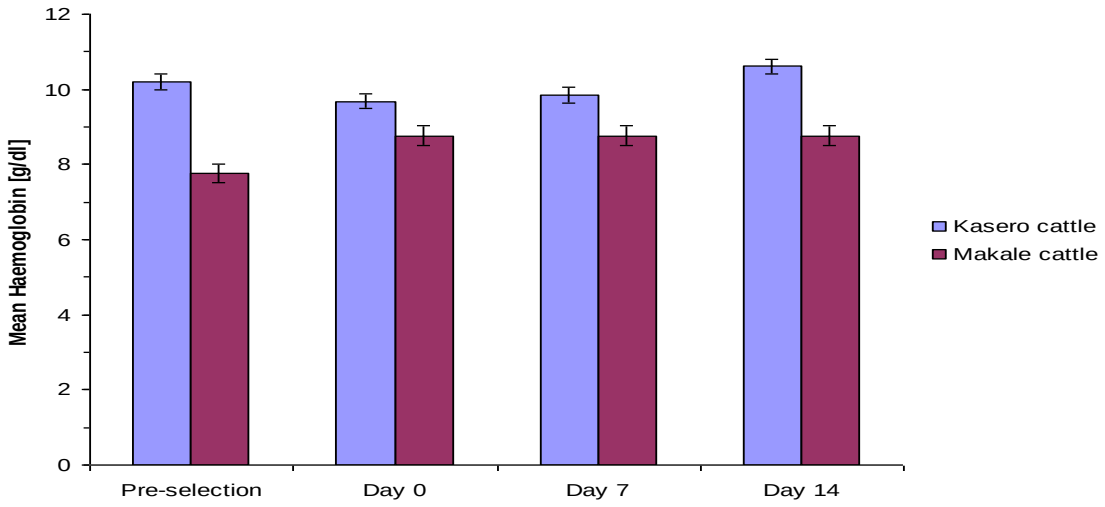

Figure 4. 3 Mean Haemoglobin levels for Kasero and Makale cattle that had motion sensors attached in Petauke District. Error bars represent standard error for the data

4.3.2 Relationship of step counts and walking activity for Petauke Cattle

Figure 4. 4 shows the relationship between the number of steps taken by individual animals in each hour as measured by motion sensors and the corresponding active measurements in Makale and Kasero Veterinary Camps during the two week study period. The active percentage measurement was the proportion of time that the animal spent being active at the particular hour of interest. As can be seen from the diagram there is a high correlation between the active percentage and the number of steps taken (Pearson correlation = 0.994, $p < 0.001$). On the basis of the high correlation, it was decided to use either the active measurement or the number of steps taken as the case may require. Furthermore, "walking" instead of "active" was going to be used throughout the study to avoid confusion in meaning.

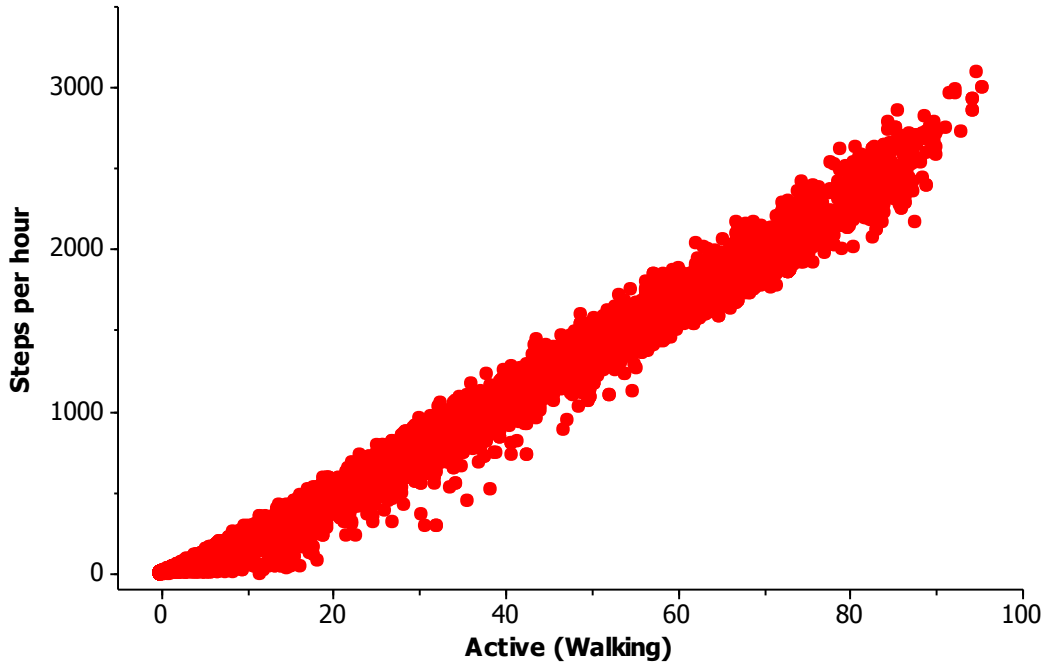

Figure 4. 4 Relationship between number of steps taken by individual animals in each hour and the percentage of time they were recorded to be "active" by motion sensors in Makale and Kasero during the two week study period.

4.3.3 Cattle movement behaviour

4.3.3.1 Step counts of cattle in Petauke District (2006/07)

Figure 4. 5 shows the mean daily steps taken by low and high haemoglobin co-grazing cattle pairs in Kasero veterinary camp during 2006/07. The mean daily number of steps taken by low haemoglobin cattle was 12,478 steps while high haemoglobin cattle had a mean number of steps of 12,729. The low haemoglobin group of cattle had the animal with both the lowest (7,409) and the highest (17,546) number of steps. A total of six low haemoglobin animals had fewer mean steps than their high haemoglobin paired partners. In only one case were

the differences between the mean numbers of steps significant with the low haemoglobin cattle taking more steps than its counterpart (Figure 4. 5).

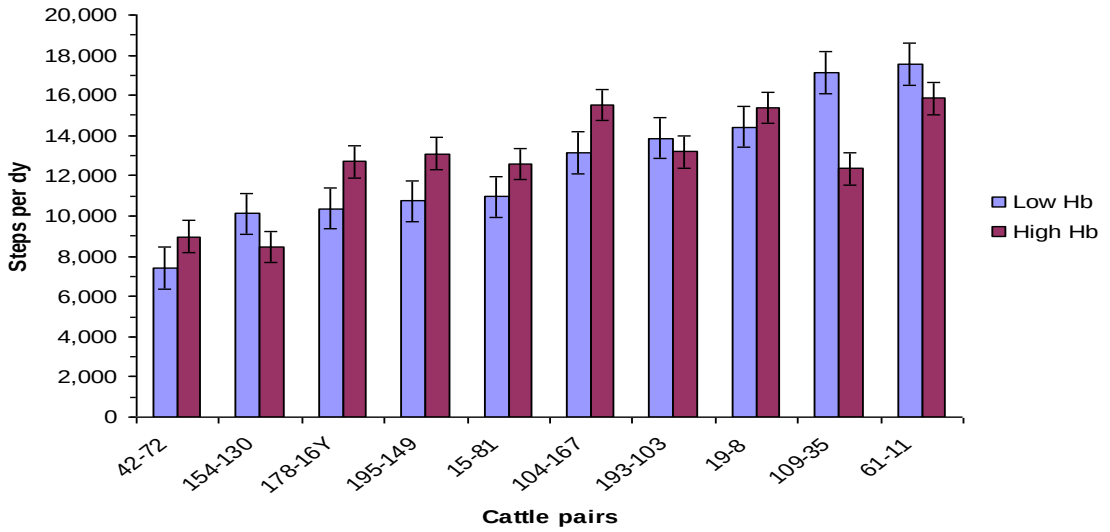

Figure 4. 5 Mean number of steps taken per day by low and high haemoglobin cattle co-grazing in pairs in Kasero. Ear tag numbers of low and high haemoglobin animals are shown. Error bars represent standard error for the data.

Figure 4. 6 shows the mean daily steps taken by low and high haemoglobin co-grazing cattle pairs in Makale veterinary camp during 2006/07. The mean daily number of steps taken by low haemoglobin cattle was 8,730 steps while the high haemoglobin cattle had a mean number of steps of 12,036. The low haemoglobin group had the animal with the lowest number of steps (3,470) while the animal with the highest number of steps (14,951) was recorded by the high haemoglobin group. A total of eight low haemoglobin animals had fewer mean steps than their high haemoglobin paired partners. In six of the cases the differences between the mean numbers of steps were significant with the high haemoglobin animals taking more steps (Figure 4. 6).

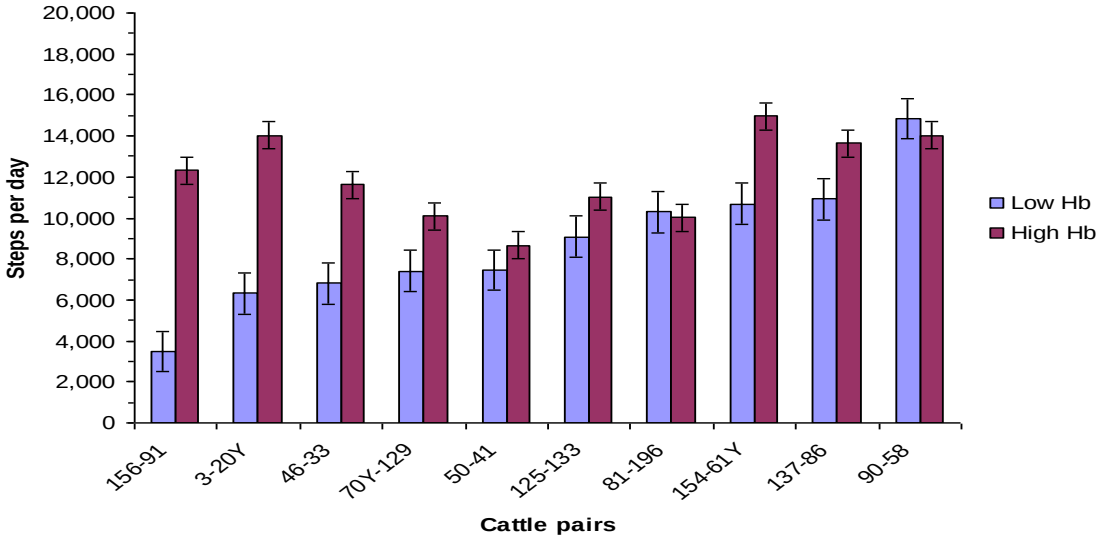

Figure 4. 6 Mean number of steps taken per day by cattle co-grazing in pairs in Makale. Ear tag numbers of low and high haemoglobin animals are shown. Error bars represent standard error for the data.

A one-way analysis of variance was used to test for differences in the number of steps taken by cattle in the high and low haemoglobin groups in both Makale and Kasero Veterinary Camps (Table 4. 3). The number of steps taken by cattle differed significantly across the four groups (F (3, 36) = 4.64, $p < 0.01$).

Table 4. 3 Number of steps taken by cattle in Petauke District in two weeks

Eartag Numbers		Makale	Makale	Kasero	Kasero
Makale (Low-High)	Kasero (Low-High)	Low	High	Low	High
46-33	42-72	95,325	162,786	103,731	125,690
50-41	154-130	104,297	121,095	141,707	118,455
81-196	178-16Y	144,033	140,431	145,152	177,803
90-58	195-149	207,922	196,235	150,584	183,506
154-61Y	15-81	149,707	209,309	153,343	176,245
125-133	104-167	127,277	154,327	183,960	217,382
3-20Y	193-103	88,381	196,158	194,227	184,939
70Y-129	19-8	103,855	140,978	201,937	215,403
137-86	109-35	152,797	191,104	239,800	173,208
156-91	61-11	48,575	172,532	245,637	222,112
Mean Steps/2 weeks		**122,217**	**168,496**	**176,008**	**179,474**

Makale high haemoglobin cattle took significantly more steps than the low haemoglobin group (paired t-test; mean difference = -3306, 95% CI [-5570, -1041], $p < 0.05$). Overall, there was no significant difference between the mean number of steps of the low and high haemoglobin cattle groups in Kasero (paired t-test; mean difference = -248, 95% CI [-1947, 1451], $p = 0.75$).

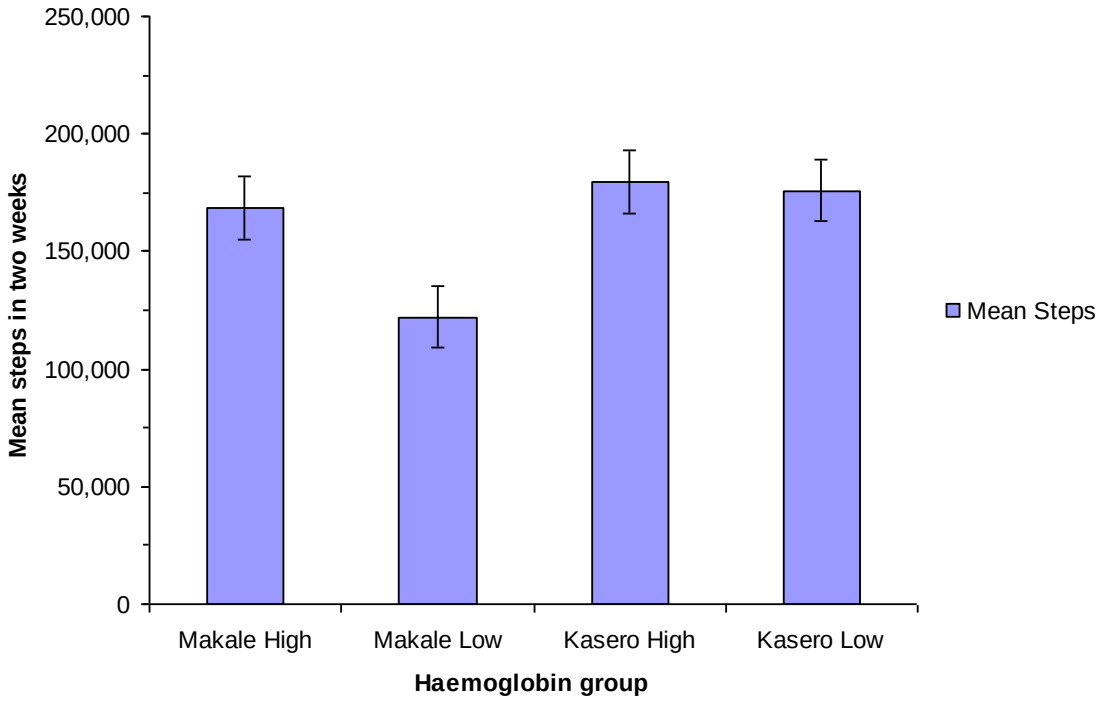

Figure 4. 7 Total number of steps taken by cattle in the high and low haemoglobin groups in Makale and Kasero. Error bars represent standard error for the data.

When comparisons between the two camps were made, it was found that Kasero cattle took significantly more steps than their counterparts in Makale (2-sample t-test; mean difference = 2221, 95% CI [315, 4127], $p < 0.05$). The main reason for the difference amongst the four haemoglobin groups appeared to be because of the significantly fewer steps taken by the Makale low haemoglobin cattle (Figure 4. 7). There were no significant differences among the other three groups.

4.3.3.2 Cattle movement behaviour profiles in Petauke District (2006/07)

Cattle movement behaviour profiles for standing, walking and lying down in Petauke District were generated from raw motion sensor data from all the forty animals over the two weeks study period.

4.3.3.2.1 Individual cattle behaviour profiles in Kasero and Makale Veterinary Camps

Cattle behaviour profiles were created for each animal in Kasero and Makale. The behaviour profiles were mean representations of individual cattle behaviour in each hour over the two week period. The movement behaviour of animals within the co-grazing pairs were investigated and compared with each other in order to detect any differences in behaviour between them.

Figure 4. 8 is a representative cattle behaviour profile showing the mean of two co-grazing pairs' behaviour during the two week period. The graph shows the mean of the three behaviours (standing, walking and lying down) of the animals. The data point for each behaviour variable at each hour is the mean behaviour of the animal during this period. Both pairs of graphs show that the walking behaviour for both animals is from after 05:00 hours in the morning until around 20:00 hours in the evening when most walking stops. For a short time around 14:00 hours, animal pairs show a marked reduction in walking activity. This reduction in walking behaviour is matched by a slight increase in lying behaviour around the same period. Apart from this slight afternoon high, lying behaviour is highest during the night time hours in between 20:00 hours and 08:00 hours. Standing behaviour is highest during the day time hours while in the night; standing behaviour is more than walking but less than lying down.

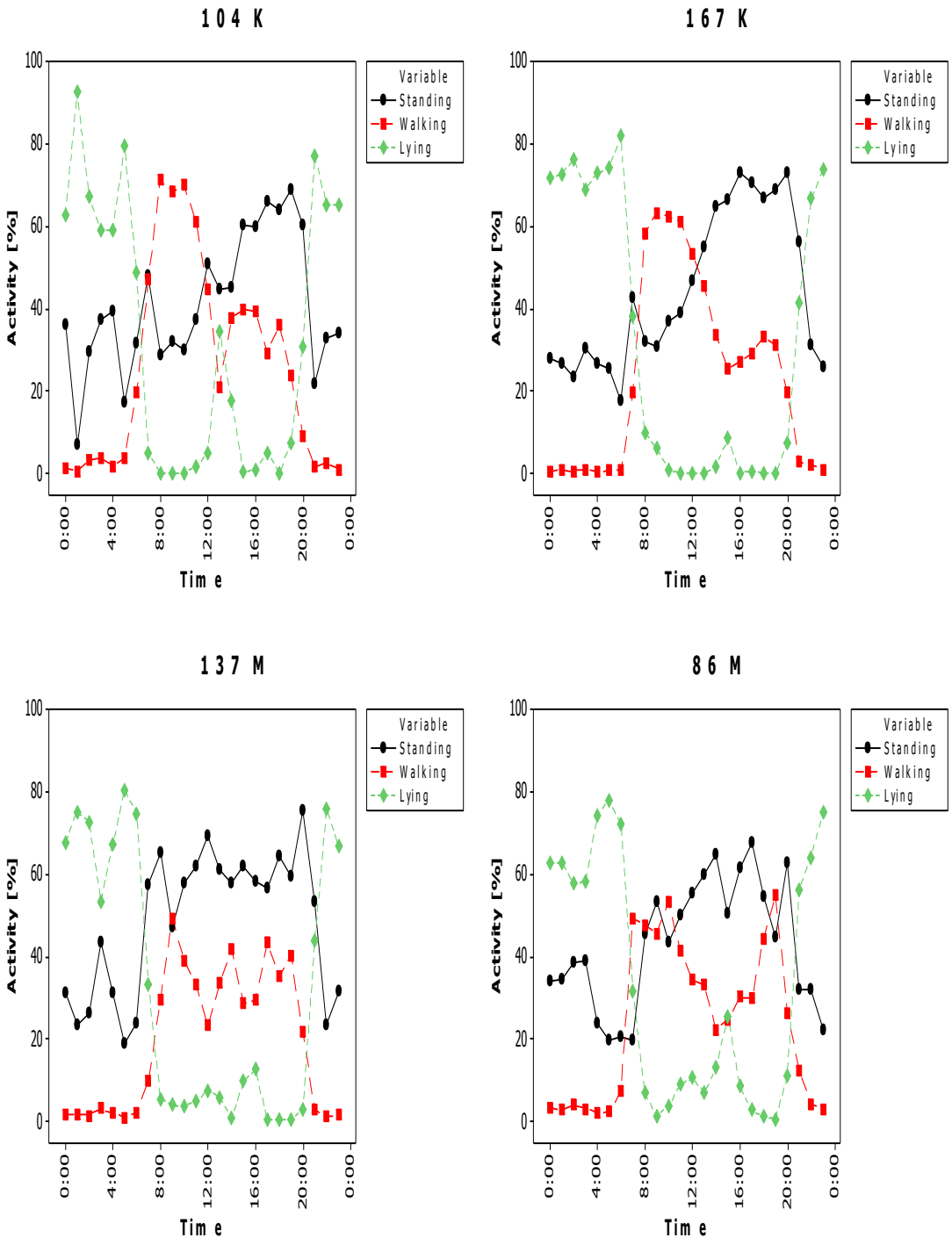

Figure 4. 8 Movement behaviour profiles of cattle pairs in Kasero (K) and Makale (M) indicating percentage of time spent standing, walking or lying down. The data point for each behaviour variable at each hour is the mean behaviour of the animal during this period. Cattle on the left are in the low haemoglobin group.

4.3.3.2.2 Cattle standing behaviour profiles

Figure 4. 9 is a boxplot of the mean standing behaviour of 40 cattle in Makale and Kasero Veterinary Camps. The figure shows that, there is always some standing activity over a 24 hour period in both Makale and Kasero. Between the night-time hours of 21:00 in the evening and 06:00 in the morning, animals in Makale appear to spend more time standing than animals in Kasero. Makale standing behaviour of animals appears to have greater variability during these hours than Kasero. Between 07:00 hours in the morning and 12:00 hours mid-day there is an increase in standing behaviour in both areas. Standing behaviour appears to be very similar in the two areas during the day up to 21:00 hours at night.

4.3.3.2.3 Cattle walking behaviour profiles

Figure 4. 10 shows the mean walking behaviour of the 40 cattle in the two areas of Makale and Kasero. Both walking behaviour profiles are very similar. Though there is very little walking between 21:00 hours in the evening and 06:00 hours in the morning, there is slightly more walking occurring in Makale. Cattle appear to start the day at around the same time (07:00 hours) in both areas. Walking behaviour continues for the rest of the day until around 21:00 hours when most walking stops. In both Makale and Kasero, there is a slight reduction in walking activity around mid-afternoon. This creates two walking "peaks" at the beginning and end of the cattle day.

4.3.3.2.4 Cattle lying behaviour profile

Mean lying behaviour for 40 Makale and Kasero cattle is shown in Figure 4. 11. The two profiles show that lying behaviour is mainly restricted to between 21:00 hours in the night and 07:00 hours in the morning. Kasero cattle spend a greater

percentage of their time lying down than cattle in Makale during these hours while there is greater lying variability for Makale cattle. There is very little lying occurring during the day, some lying behaviour still occurs in both areas. In Makale, there is a gradual increase in lying behaviour from around 09:00 hours until it peaks around 14:00 hours to 15:00 hours. Kasero animals exhibit sporadic lying behaviour throughout the morning but have a peak at around 14:00 hours to 15:00 hours.

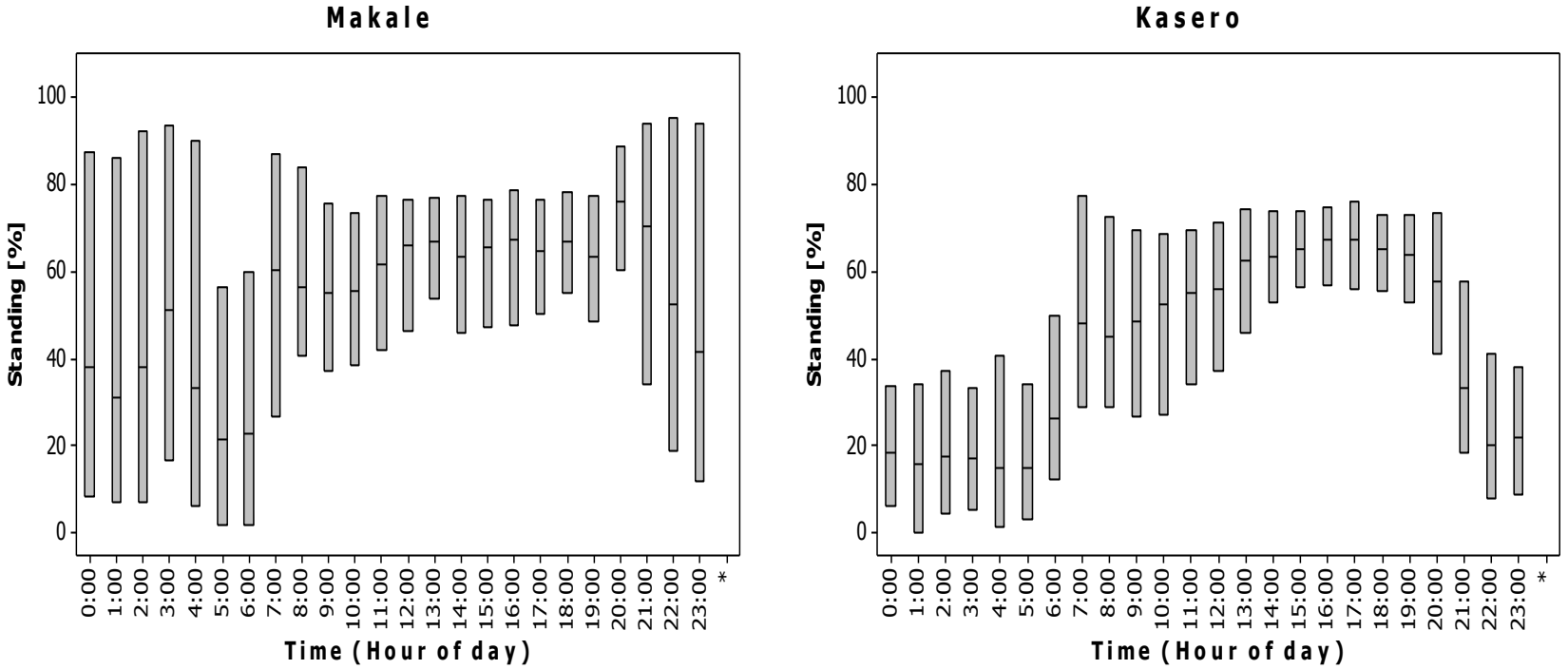

Figure 4. 9 Mean standing behaviour profiles of cattle in Makale and Kasero for each hour of the day during the two week period. Each box indicates the lower and upper quartiles representing the points below which 25 % and 75 % of the observations lie respectively. The median value is shown within each box as a horizontal line. This is the value below which half, and above which half, the observations lie.

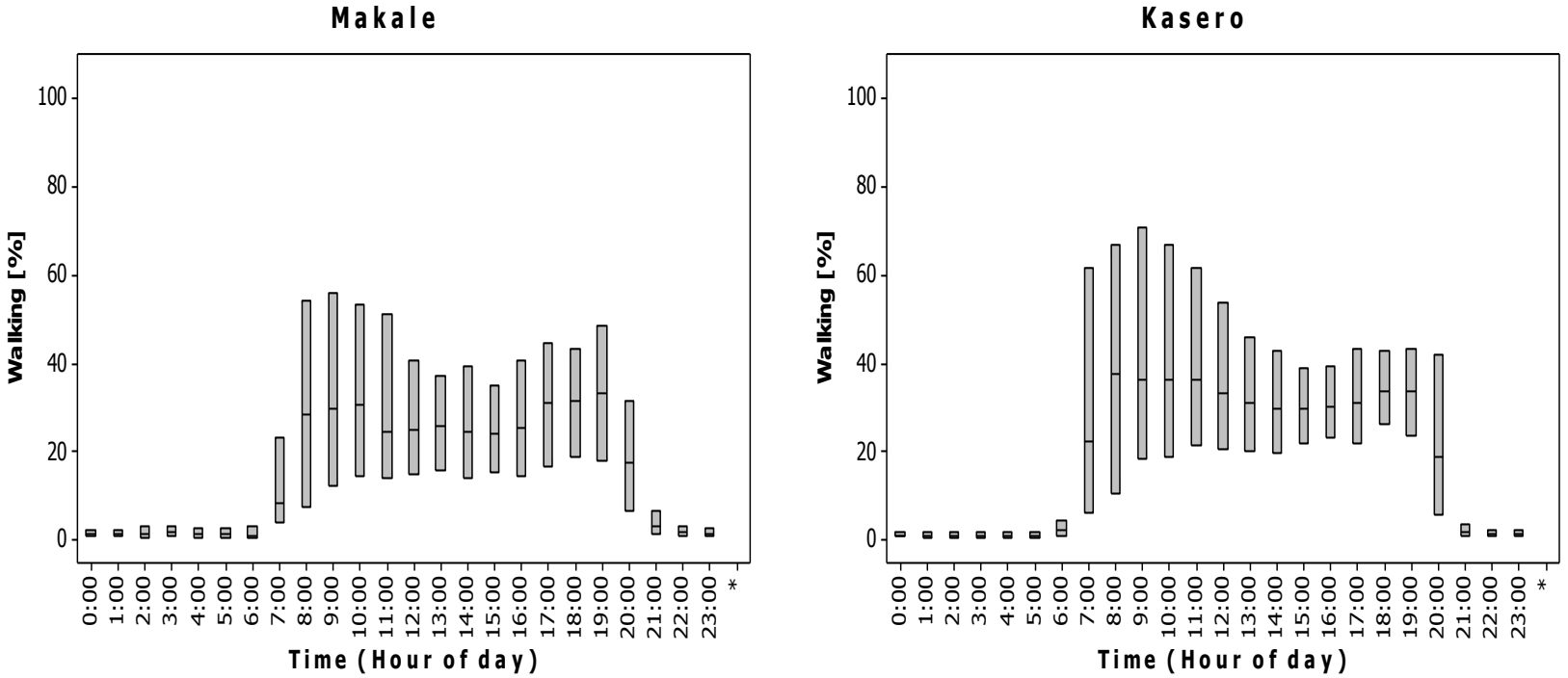

Figure 4. 10 Mean walking behaviour profiles of cattle in Makale and Kasero for each hour of the day during the two week period.

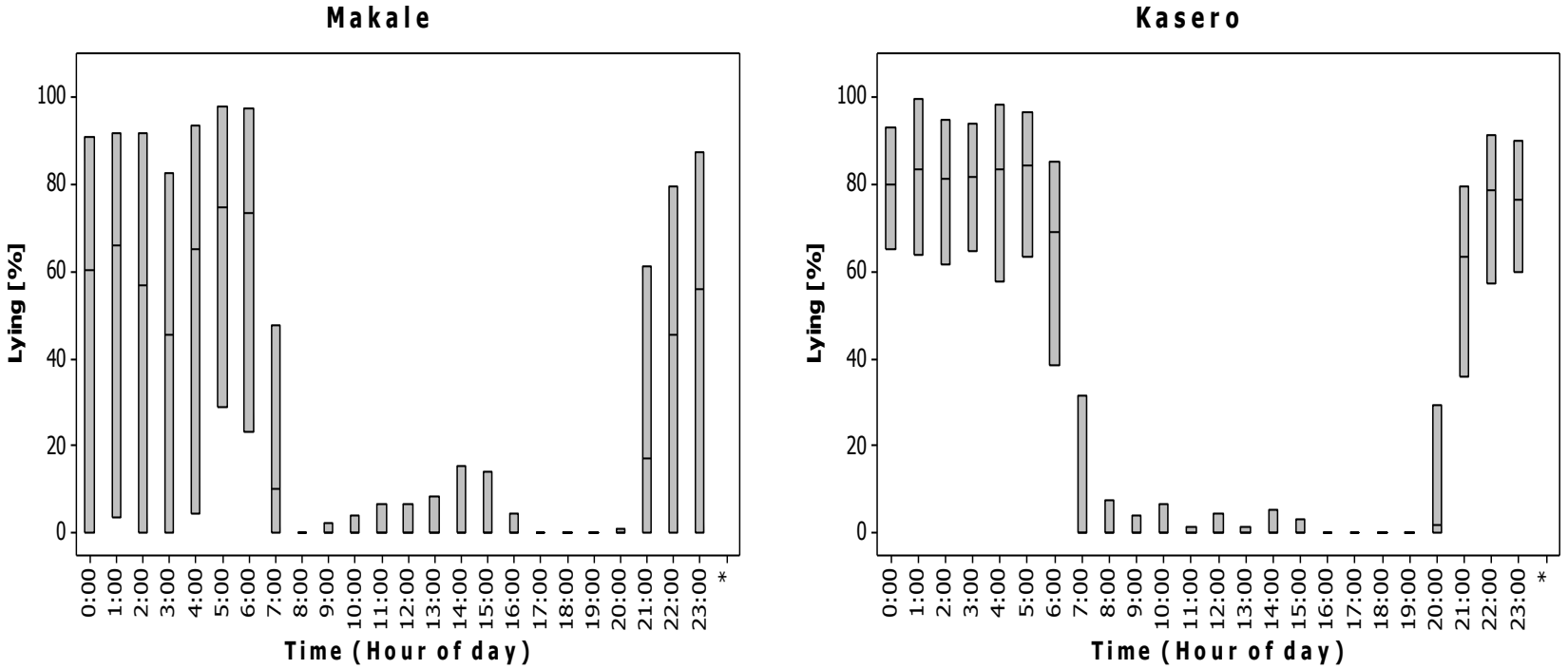

Figure 4. 11 Mean lying behaviour profiles of cattle in Makale and Kasero for each hour of the day during the two week period.

4.3.3.3 Mean cattle time budgets in Kasero and Makale

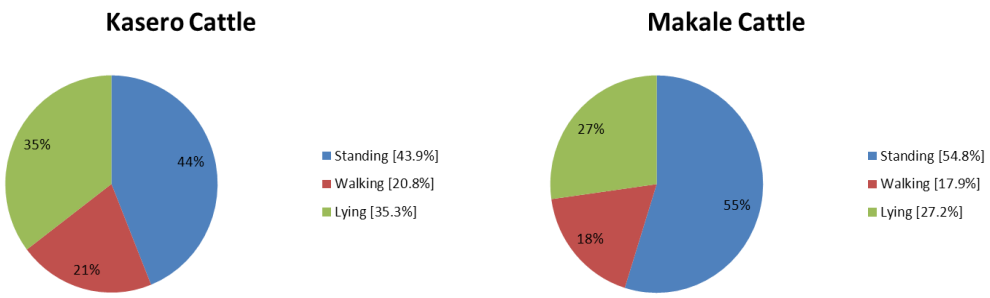

Figure 4. 12 Kasero and Makale time budgets. Pie charts show the mean time cattle spent standing, walking and lying down in the two week period.

Kasero cattle spent a total of 44% of their time standing over the two week study period (Figure 4. 12). The animals in this area spent 21% of their time walking while 35% was spent lying down. Makale cattle spent more time standing (55%) than Kasero cattle (Figure 4. 12). Cattle in Makale veterinary camp also spent a total of 27% of their time lying down and 18% walking.

The difference in mean time spent standing for cattle that had motion sensors attached in the two areas was found to be highly significant (2-sample t-test; estimate for difference of means = -10.9, 95 % CI [-11.9, -10.0], $p < 0.001$) with Makale animals spending more time standing. There was also a highly significant difference between the mean time spent lying down by cattle in the two areas (2-sample t-test; estimate for difference of means = 8.1, 95 % CI [6.9, 9.3], $p < 0.001$) with Kasero animals spending more time lying down. There was also a highly significant statistical difference between the mean time spent walking by cattle in the two areas (2-sample t-test; estimate for difference of means = 2.8, 95 % CI [2.1, 3.6] $p < 0.001$) with Makale cattle taking fewer steps than their counterparts in Kasero.

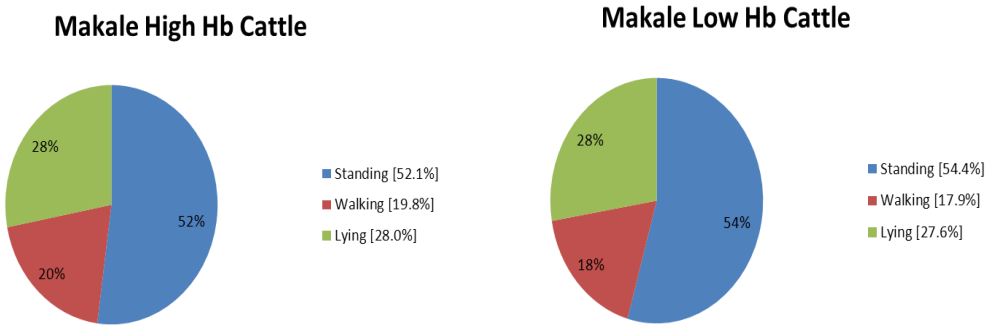

Figure 4. 13 Makale time budget for the high and low haemoglobin groups. Figure shows the mean time cattle spent standing, walking and lying down in the two week period.

Time budgets for Makale animals that had high and low haemoglobin levels are shown in Figure 4. 13. Low haemoglobin animals spent more time standing than animals in the high haemoglobin group and this difference was found to be highly statistically significant (2-sample t-test; estimate for difference of means = 2.3, 95 % CI [1.2, 3.4] $p < 0.001$). High haemoglobin animals spent more time walking than animals in the low haemoglobin group and this difference was found to be highly statistically significant (2-sample t-test; estimate for difference of means = -1.9, 95 % CI [-2.7, -1.2] $p < 0.001$). There was no statistical difference between the mean time spent lying down by cattle in the two groups (2-sample t-test; estimate for difference of means = -0.4, 95 % CI [-1.7, 0.91] $p = 0.56$) over the two week period.

4.3.4 Body condition scores of cattle in Petauke District

The mean body condition score of all cattle in Kasero was 4.82 while that for Makale was 4.76. There was no significant difference between the condition scores of Kasero and Makale cattle (2-sample t-test; estimate for difference of means = 0.05, 95 % CI [-0.11, 0.22] $p = 0.55$). The great majority of animals fell into the medium category (89.1% for Makale and 94.8% for Kasero). Makale had

10.4% of animals in the lean category and 0.5% in the fat category. Kasero had 3.8% of animals in the lean category and 1.4% in the fat category.

4.3.5 Principal Components Analysis

The cattle behaviour profiles were successful in visually showing the mean cattle movement behaviour in the two study sites. A more powerful tool was needed to explore the cattle movement data more quantitatively and establish any associations between the two areas or between individuals within the areas. For this purpose, a principal components analysis was used.

A typical principal components routine in a standard software package will have the coefficients (or loadings) of each principal component; the values of each individual on each component (known as the scores on each component) and the variances of the scores for each component (known as the eigenvalues). The output is arranged so that the component with the largest variance (eigenvalue) comes first; the one with the next largest variance comes second, and so on. The best two-dimensional configuration for viewing the data is given by the first two principal components, so that the scatter diagram obtained by plotting the scores on the first two components against each other provides a method to allow for examination of patterns. However, the scores on the third, fourth and succeeding components may also need to be considered if the effective dimensionality is greater than two (Krzanowski, 2007).

Determining the effective dimensionality is generally addressed by looking at the variances associated with each principal component. The sum of the variances for all components gives the total variance for the data. So the ratio of the variance of each component to this total gives the proportion of overall variance

accounted for by that component, and the cumulative sums of this proportion give the proportions accounted for by the first, the first two, the first three components, and so on (Krzanowski, 2007). A common way of judging the effective dimensionality is to specify it as the number of components required to pass a suitably large proportion – typically around 0.8. This means that around 80 % of the variability of all data is contained within this dimensionality, and the remaining 20 % is small enough to be ignored. In many practical applications such a proportion is reached at fairly low dimensionalities, so considerable simplification of data structure can be achieved (Krzanowski, 2007). Other methods of determining dimensionality include choosing those principal components with eigenvalues above one or using the scree plot as described below.

Most implementations of PCA will generate a scree plot which can be used to determine the number of factors which should be extracted for further analysis. The scree plot is therefore a plot of the eigenvalue associated with a principal component versus the number of the component. The scree plot is used to judge the relative magnitude of the eigenvalues. The cut off point for factor extraction is placed at the "elbow" of the graph or at a point where the eigenvalue is above one.

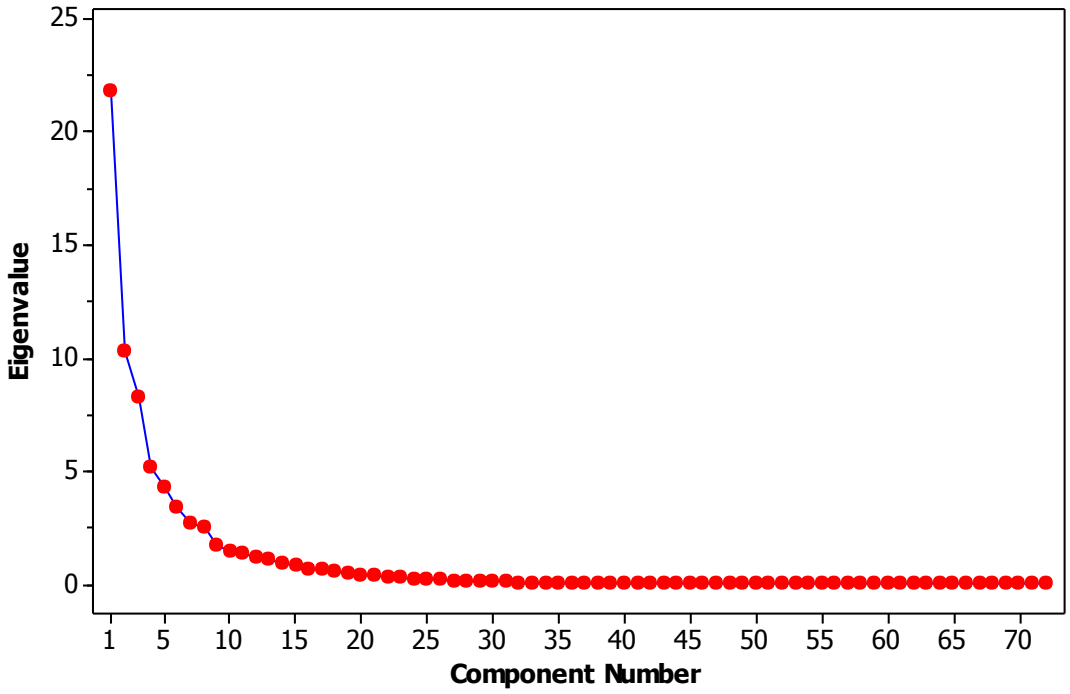

Figure 4. 14 Scree Plot based on 72 variables expressing cattle behaviour in Petauke District

4.3.5.1 Score plots of Makale and Kasero cattle

The principal components analysis for studying the cattle motion sensor data was carried out for the two weeks that motion sensors were attached on all of the 40 animals in the study in Makale and Kasero Veterinary Camps. The first four principal components had a cumulative eigenvalue proportion of 0.63 indicating that these principal components accounted for over 60% of the variance.

Table 4. 4 Principal component eigenvalues and proportions

PC	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Eigenvalue	21.811	10.329	8.242	5.180	4.266	3.386	2.666	2.521	1.712
Proportion	0.303	0.143	0.114	0.072	0.059	0.047	0.037	0.035	0.024
Cumulative	0.303	0.446	0.561	0.633	0.692	0.739	0.776	0.811	0.835

PC	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Eigenvalue	1.445	1.326	1.195	1.117	0.934	0.798	0.682	0.631	0.584
Proportion	0.020	0.018	0.017	0.016	0.013	0.011	0.009	0.009	0.008
Cumulative	0.855	0.873	0.890	0.906	0.918	0.930	0.939	0.948	0.956

Score plots were generated from the PCA to determine the animal's relative positions in two dimensional space. The score plots were generated from the first four principal components (PC) that accounted for 63.3 % of the cumulative variability which was almost two-thirds of overall data variability. The first PC was the major contributor and accounted for 30.3 % of the overall variability in the data. Principal components two, three and four accounted for 14.3 %, 11.4 % and 7.2 % respectively. Score plots were generated from the PCA to illustrate the animals' relative positions in two dimensional space. Plotting the first four components against each other offered the best picture to examine for complex patterns within the data. From the first four PCs, a total of six score plots were plotted (Appendix 5). Figure 4. 15 shows a score plot of the first and second PC while Figure 4. 16 shows a score plot of the first and third PC.

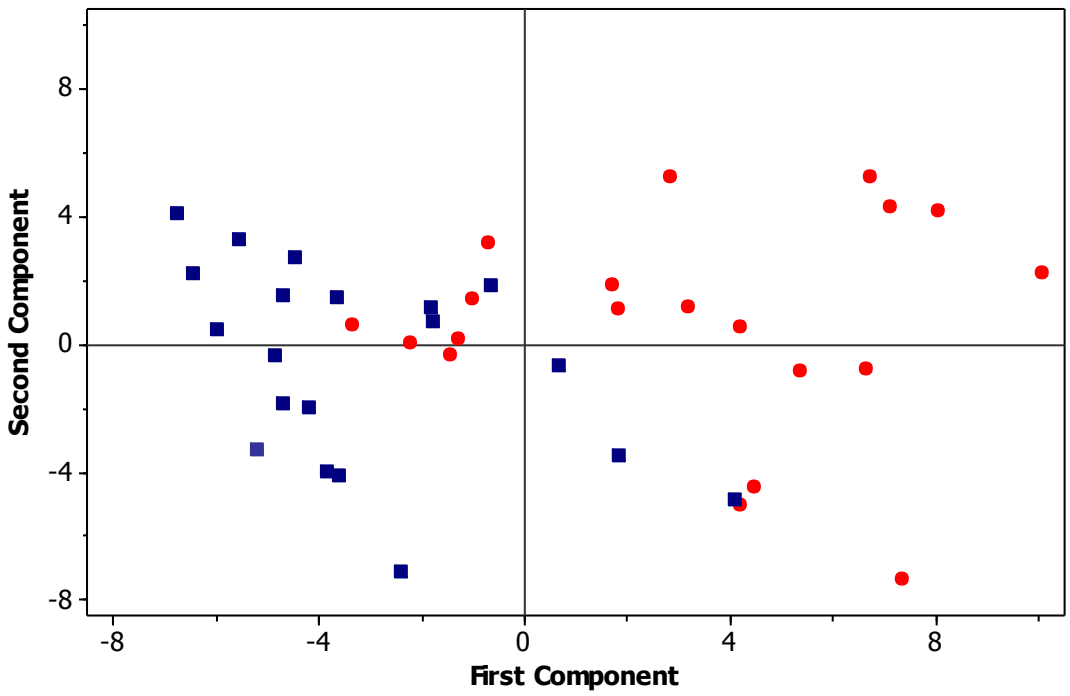

Figure 4. 15 Score Plot of the first and second Principal Components resulting from the PCA of motion sensor data of cattle in Kasero (■) and Makale (●) veterinary camps

Examining Figure 4. 15 and Figure 4. 16 shows that animals in the two areas are clearly divided in to two clusters based on certain characteristics of their behaviour. Figure 4. 16 seems to better show the differences between animals in Kasero and Makale with the Kasero cluster having all but three animals represented within the cluster. The Kasero animals had high mean haemoglobin values of 10.4 g/dl (Chapter 6, Section 6.4.1.1) and are more closely clustered than the Makale animals which had lower haemoglobin values of 9.4 g/dl (Chapter 6, section 6.4.1.1) and were more sparsely spread.

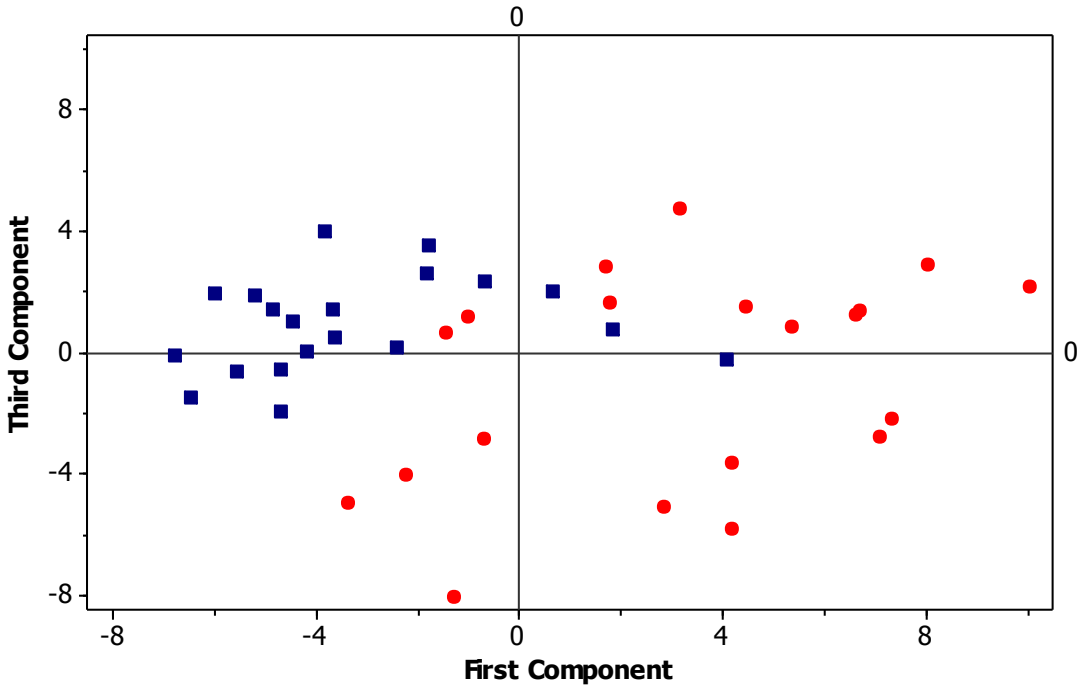

Figure 4. 16 Score Plot of the first and third Principal Components resulting from the PCA of motion sensor data of cattle in Kasero (■) and Makale (●) veterinary camps

Further analysis of the data was performed by retaining the Principal Components that had eigenvalues greater than one. Table 4.4 indicates that there were thirteen PCs that had eigenvalues above one. The 13 PCs that had eigenvalues greater than one accounted for over 90 % of the total variability in the data. The first thirteen Principal Components and their loadings for the

standing, walking and lying variables are displayed in Table 4. 5**.** Only each variable's highest (absolute) loadings are shown in the table and interpretations were based on these loadings. The complete loadings for PCA results in this thesis are shown in appendices 1 and 2. The variables loading on Principal Component 1 fell into two groups. The first group had positive Principal Component loadings and highlighted standing behaviour in the night and early morning between the hours of 21:00 and 04:00. The second group had negative Principal Component loadings and highlighted lying behaviour in the night and early morning between the hours of 21:00 and 05:00. Because of the bipolarity in algebraic signs, it appears that the first component, which was the highest contributing factor at 30.3 %, was contrasting standing behaviour with lying behaviour in the night and early morning hours (Figure 4. 17).

Table 4. 5 Principal Components Loadings for Standing, Walking and Lying behaviour in Petauke District (2006/07). Only each movement behaviour variable's highest (absolute) loadings are shown.

	Principal Component		*Principal Component*		*Principal Component*	
Time	Standing	Loading	Walking	Loading	Lying	Loading
0:00	1	**0.179**	8	**0.233**	1	**-0.18**
1:00	1	**0.174**	6	**0.247**	1	**-0.174**
2:00	1	**0.181**	12	**-0.232**	1	**-0.178**
3:00	1	**0.191**	4	**-0.222**	1	**-0.189**
4:00	1	**0.171**	11	**-0.264**	1	**-0.173**
5:00	11	**0.199**	7	**0.312**	1	**-0.179**
6:00	9	**0.239**	5	**-0.291**	4	**-0.235**
7:00	13	**-0.233**	12	**0.191**	9	**-0.345**
8:00	11	**-0.168**	2	**0.205**	11	**0.299**
9:00	10	**-0.189**	2	**0.208**	8	**0.244**
10:00	4	**-0.196**	2	**0.194**	2	**-0.2**
11:00	3	**0.183**	2	**0.208**	2	**-0.233**
12:00	12	**0.167**	2	**0.196**	2	**-0.24**
13:00	7	**0.187**	13	**0.315**	13	**-0.236**
14:00	7	**0.251**	11	**-0.307**	11	**0.299**
15:00	11	**-0.241**	5	**-0.263**	3	**-0.226**
16:00	6	**0.275**	5	**-0.264**	6	**-0.186**
17:00	6	**0.259**	5	**-0.26**	5	**0.202**
18:00	9	**-0.272**	9	**0.261**	5	**0.221**
19:00	9	**-0.313**	6	**-0.21**	11	**-0.242**
20:00	13	**-0.385**	7	**0.329**	13	**0.337**
21:00	1	**0.173**	7	**0.385**	1	**-0.168**
22:00	1	**0.178**	6	**0.251**	1	**-0.178**
23:00	1	**0.18**	13	**0.327**	1	**-0.181**

Principal Component 2 showed a positive loading for walking behaviour between the hours of 08:00 and 12:00, and a negative loading for lying behaviour between the hours of 10:00 and 12:00. This component had an overall contribution to data variability of 14.3 % and appears to reflect the walking behaviour in the early morning hours contrasted with late morning to midday lying behaviour (Figure 4. 17).

Table 4. 6 Principal Components and movement behaviour variables in Petauke District

	PC1 (30.3%)	PC2 (14.3%)	PC3 (11.4%)	PC4 (7.2%)
Standing	21:00–04:00hrs	-	11:00hrs	10:00hrs
Walking	-	08:00-12:00hrs	03:00hrs	-
Lying	21:00-05:00hrs	10:00-12:00hr	15:00hrs	06:00hrs

Principal Component 3, which contributed 11.4 % of overall variability, was purely a component that contrasted standing, walking and lying behaviour at specific hours of the day (Figure 4. 17).

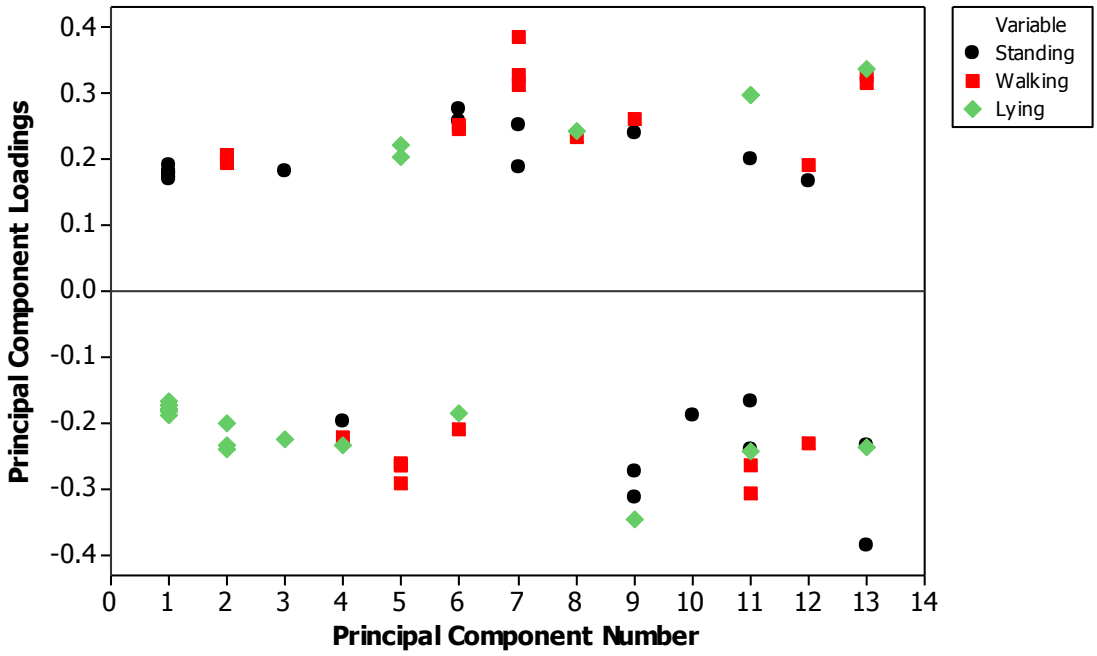

Figure 4. 17 Relationship of principal components (PC) and their corresponding loadings from the analysis of standing, walking and lying behaviour in Petauke District

It contrasted standing behaviour in the morning at 11:00, walking behaviour at 03:00 and lying behaviour at 15:00. Principal Component 4 had overall variability contribution of 7.2 % and contrasted standing and lying behaviour in the

morning hours of 10:00 and 06:00 respectively. The remaining PCs had an overall variability contribution of less than 6 % and they each involved different combinations of the three variables over the 24 hour period.

4.3.5.2 Distances between motion sensor cattle pairs on score plots

Co-grazing pairs of cattle in Petauke were identified on the score plots and their relative positions to each other were linked and compared to other co-grazing pairs. Figure 4. 18 shows a score plot of the first and second PC with the data labels representing the ear tag numbers of Makale and Kasero cattle. The figure also shows the relative positions of co-grazing pairs linked by drawn lines that show the relationship between the pairs. Using the loadings for principal components 1 and 2, the Euclidian distances between two co-grazing pairs were calculated in score units using the first component as the x co-ordinate and the second component as the y co-ordinate for the position of each animal in the pair.

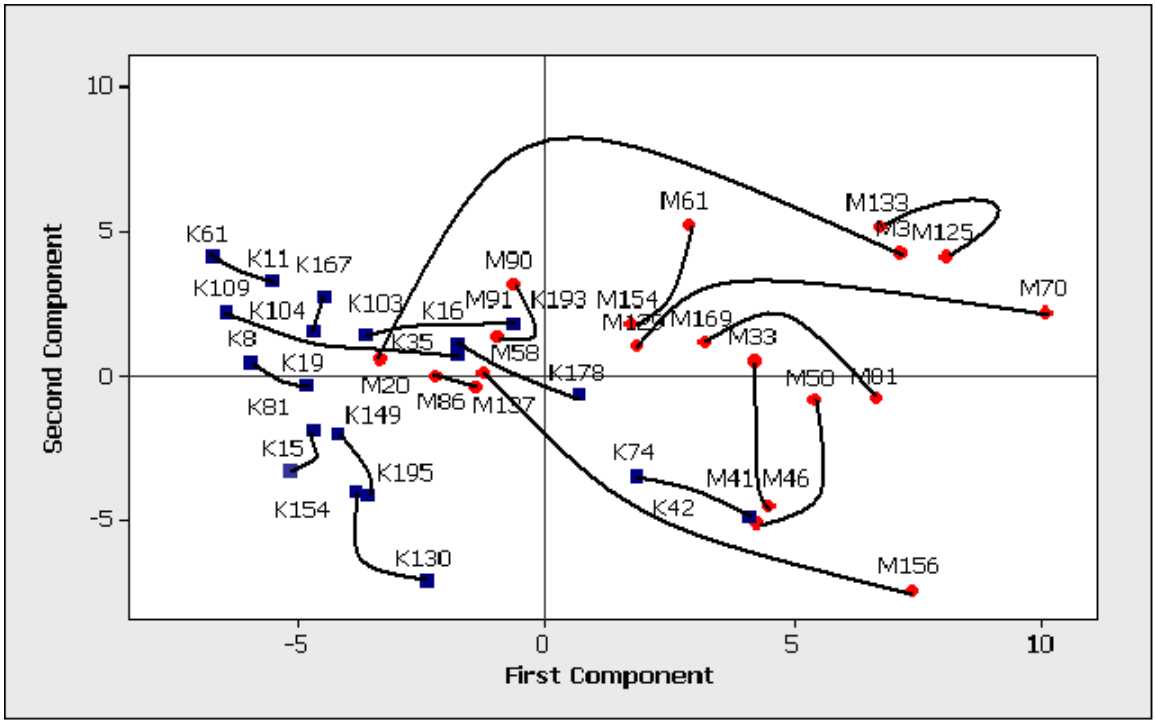

Figure 4. 18 Score Plot of the first and second Principal Components showing co-grazing pairs of cattle linked with hand drawn lines in Kasero (■) and Makale (●) Veterinary Camps

The distances calculated in score units between co-grazing motion sensor wearing cattle in Makale and Kasero Veterinary Camps are shown in Table 4. 7 and Figure 4. 19. A 2-sample Wilcoxon rank sum test (Mann-Whitney test or 2 -sample rank test) to test the equality of the two population medians was conducted. A significant difference was found between Makale and Kasero cattle pairs in terms of their score unit distances (median for the ordered data for Kasero = 2.62, median for Makale = 4.19; 95.5% CI for the difference in population medians [-6.17, -0.11] test statistic, W = 77; $p < 0.05$).

Table 4. 7 Distances (in score units) between cattle pairs on a score plot generated from the first and second principal components

Kasero Pairs	*Pair* Number	*Distance* (score units)	*Makale* Pairs	*Pair* Number	*Distance* (score units)
K35 - K109	1	4.90	**M33 - M46**	11	5.01
K8 - K19	2	1.34	**M41 - M50**	12	4.44
K81 - K15	3	1.62	**M169 - M81**	13	3.95
K11 - K61	4	1.49	**M58 - M90**	14	3.09
K74 - K42	5	2.60	**M61 - M154**	15	3.44
K103 - K193	6	2.95	**M133 - M125**	16	1.91
K149 - K195	7	2.64	**M20 - M3**	17	11.07
K16 - K178	8	3.03	**M129 - M70**	18	8.29
K167 - K104	9	1.15	**M86 - M137**	19	0.90
K130 - K154	10	3.50	**M91 - M156**	20	12.28

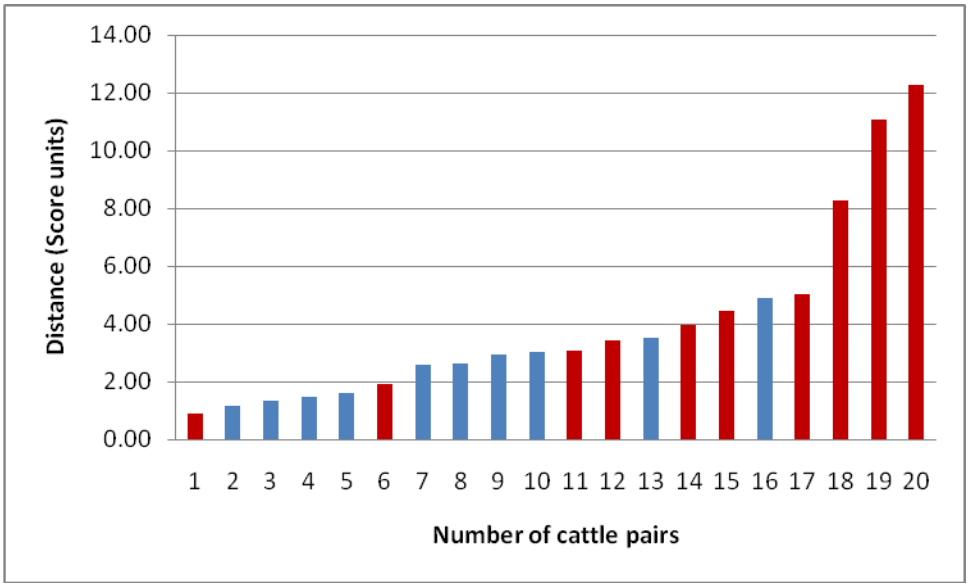

Figure 4. 19 Score plot distances for Kasero (blue) and Makale (red) cattle pairs The score plot distance is the distance (in score units) between the two animals in the pair based on the loadings of principal components 1 and 2.

4.4 Discussion

4.4.1 Suitability of motion sensors for studying cattle movement behaviour in Africa

The results of this study clearly indicated that two-dimensional motion sensors could be used to study cattle movement behaviour under a traditional African livestock management system. The technology was accepted by both the farmers and their animals. The farmers were very co-operative throughout the whole programme as they understood that the long term goals of the study would benefit their community. Most of the cattle owning farmers attended the meetings or sent their representatives. Cattle were presented for clinical examination and motion sensor attachment when requested. Farmers were given and accepted the responsibility for monitoring the motion sensors whilst they were attached to their animals. As a result, no motion sensor was stolen or lost during the study. This may have been because, it was made very clear during the farmer sensitisation meetings that there were no parts inside the sensors that could be reused to make items such as simple radios (a worry raised by local veterinary staff). The farmers accepted the technology to be used on their animals as they were fully informed of what was happening from the beginning. It has been observed that when people understand a control option and its benefits, they are likely to respond positively (Tatchell, 1981). Animals appeared not to be too bothered by attachment of the motion sensors to their hind limbs either. As precaution of preventing too much discomfort, the Velcro straps attaching the sensors were not attached too tight around the animals' legs. Additionally, the sensors were changed to the other leg after a week of attachment.

4.4.2 Step Counts of Cattle in Petauke District

Two-dimensional motion sensors were used successfully to record the step counts of animals in the motion sensor study in Petauke District of the Eastern Province. Cattle in Kasero were found to have higher mean step counts than Makale cattle. This difference in the mean step counts of cattle in the two areas was found to be significant ($p < 0.05$). Kasero animals were also found to have significantly higher haemoglobin values than Makale cattle ($p < 0.05$). It is suggested that Makale animals had lower haemoglobin levels because of the pathogen present in this area (*T. congolense*, savannah type) which causes a much more severe type of anaemia than an infection of *T. parva* in cattle (Bengaly et al., 2002; Masumu et al., 2006c; Taylor, 1930).

Kasero cattle that had motion sensors attached showed no significant difference between step counts of high and low haemoglobin groups ($p = 0.75$) during the two week study period. This is despite there being a significant difference in the mean haemoglobin values between the two groups ($p < 0.05$). This may have been because both the low and high haemoglobin groups in Kasero had mean haemoglobin levels above normal levels of 8g/dl. This may have resulted in all cattle in the area behaving in a normal manner in terms of movement behaviour and showing no difference between the high and low groups because they had above normal haemoglobin levels and were not anaemic.

In Makale, there was a significant difference between step counts of the high and low haemoglobin cattle ($p < 0.05$) during the two week study period. There was also a significant difference between the mean haemoglobin values of the high and low groups ($p < 0.05$). Low haemoglobin cattle took fewer steps than high

haemoglobin cattle. Makale had a larger proportion of animals having haemoglobin values below 8g/dl. As a result, when selecting animals into the high and low haemoglobin groups, it was easier to find animals at both ends of the scale while still fulfilling the other selection criteria. As a result, low haemoglobin cattle had mean haemoglobin of 6.7 g/dl while high haemoglobin cattle had mean haemoglobin of 12.2 g/dl. Because the two groups were distinctly divided on the basis of circulating haemoglobin levels, this might have led to the difference in movement behaviour with high haemoglobin animals having higher step counts than those in the low group.

There was a significant difference between Makale and Kasero mean step counts ($p < 0.05$). Kasero cattle had higher step counts, higher mean haemoglobin values and higher proportion of animals infected with *T. parva*. Makale had lower step counts, lower mean haemoglobin values and a higher proportion of animals were infected with *T. congolense*. Infection with *T. congolense* (savannah type) causes a severe anaemia in cattle**.** This may have been the cause of the low haemoglobin values in this area resulting in the possible reduction of movement activity as cattle might have been physically weaker than their counterparts in Kasero.

4.4.3 Cattle Movement Behaviour in Petauke District

Principal Component Analysis (PCA) is a statistical method that is used to reduce multidimensional datasets to lower dimensions and to identify new, meaningful, hidden differences between the generated clusters. A PCA was used in this study to investigate which of the three variables (standing, walking or lying down) was more important in determining the overall variability of the

motion sensor data over a twenty four hour period. The PCA makes it possible to reduce the number of variables while maintaining as much of the original information as is possible. The original set of variables can then be reduced to a smaller set that accounts for most of the variance in the data (William R. Dillon, 1984). The purpose of Principal components analysis is to determine factors (i.e., principal components) in order to explain as much of the total variation in the data as possible with as few of these principal components as possible (William R. Dillon, 1984).

The result of the PCA is represented by two dimensional plots called the score plots. The score plot is based on the PCA and shows similarities and differences among the cases. Cases with similar patterns are clustered closely within the plot. In this study two-dimensional score plots (Figure 4. 15, Figure 4. 16, Figure 4. 18) and loading profiles (Table 4. 5) of the principal components (PC) were used to visualise the relative contribution of individual movement behaviour variables to the clustering of the different animals. Score plots from the PCA of Makale and Kasero cattle revealed separate clusters of cattle from the two areas reflecting underlying differences between their movement behaviours (Figure 4. 15).

The Principal Components loading profiles indicated that clustering was mainly based on the differences in night time standing and lying behaviour in the two areas (Table 4. 6, Table 4. 5). Cattle movement behaviour profiles revealed that cattle in Kasero spent more time lying down than Makale cattle between the hours of 21:00 hours at night and 06:00 hours in the morning, conversely Makale cattle spent more time standing. These results appeared to indicate that the two study sites differed in terms of their cattle movement behaviour.

The PCA analysis and behaviour profiles both allowed comparison of cattle movement behaviour in Makale and Kasero at different times of the day. Both analyses showed that there are night time differences in standing behaviour between the two study sites. Makale animals stood for longer periods of time during the night than cattle in Kasero ($p < 0.05$). Conversely, Kasero cattle spent more time lying down than their Makale counterparts ($p < 0.05$).

In the dairy industry, differences in standing activity have been associated with lameness in cattle (Cook et al., 2005). Farmers in both Kasero and Makale reported footrot (interdigital phlegmon, foul in the foot) as one of the important cattle diseases in the area (Chapter 3, Section 3.3.3). The kraals where cattle are kept at night play an important role in the management of cattle. Studies conducted in the Gambia concluded that kraals where cattle are kept for long periods of time, especially in the rainy season, become heavily contaminated with dung and mud making ideal conditions for transmission of disease pathogens (Kaufmann et al., 1993). This may be the reason that foot rot was reported as an important disease in the area. Footrot is associated with lameness in cattle (Merck Veterinary Manual, 2006) and may explain why cattle in one area spent more time standing up than in another area. Large increases in standing time have been associated with increasing severity of lameness (Cook et al., 2005). Since Makale cattle spent more time standing, they might have had a higher proportion of lame animals although this was not observed by the investigators.

Cattle lying behaviour has been used as a measure of cattle well-being in the dairy industry (Cook et al., 2005; Robert et al., 2009). The differences in night time lying/standing behaviour might also have been due to the conditions of

kraals where the animals slept during the study period. Due to limitations on the number of motion sensors available, the study was initially carried out in Kasero before being repeated in Makale. The rains had started during the study in Kasero but intensified as the study moved to Makale (Personal observation). This difference in time of carrying out the study meant that Makale had more rains which might have explained why animals were standing more because the ground had dung and was wet and muddy making it less appealing for lying down and creating ideal conditions for transmission of pathogens (Kaufmann et al., 1993).

The walking behaviour profile for both areas was not very different. Animals in both areas started the day at around 07:00hrs and stopped almost all walking activity by 21:00hrs. Overall, there was a slight decrease in walking behaviour between 14:00hrs and 15:00hrs. This corresponded with a slight increase in lying behaviour around the same period. This appeared to be an indication that animals were resting at this time of day. Animals around this time of the day were seen resting under the shades of trees as it was very hot during this time of day (Personal observation). The body condition scores did not significantly differ between Kasero and Makale cattle. This may have been because of the abundant availability of grass for grazing that is common during the rainy season.

The two-dimensional motion sensors successfully recorded the step counts, proportion of time spent standing, walking and lying down in Petauke District. Motion sensors have been evaluated to monitor and classify animal behaviour successfully by other researchers (Robert et al., 2009; Walker et al., 1985). The original 72 dimensional data was reduced by PCA to thirteen accounting for 90.6 % variability of the data. The first four PCs together accounted for 63.3 % of the

overall variability in the data (Table 4. 4). This shows that only four of the PC's were able to account for more than half of the variability in the data. Variability of data was mainly due to differences in the animals standing and lying behaviours in Makale and Kasero.

IceTag™ two-dimensional motion sensors successfully quantified cattle movement behavioural characteristics in a traditionally managed crop-livestock farming system in the Eastern Province of Zambia. Despite being used during adverse wet and muddy weather conditions in the rainy season, the motion sensors recorded the standing, walking, lying and step counts of cattle with minimal labour and caused no major technical problems during the data capture phase. It has been observed that sampling cattle behaviours demands a high degree of labor, equipment, and time (Mitlohner et al., 2001; Robert et al., 2009). The use of motion sensor technology in the study of cattle movement behaviour is relatively new and was successfully used in this study to collect movement data on traditionally managed cattle in sub-Saharan Africa.

The study showed that Kasero cattle had significantly higher step counts and haemoglobin levels than their Makale counterparts. The results suggest that cattle with high haemoglobin will be physically more active than cattle with lower levels of haemoglobin. In addition to the differences in step counts between animals from the two areas, the two study sites also differed in terms of their cattle movement behaviour. The differences were mainly based on the differences in night time standing and lying behaviour in the two areas which were statistically significant. Kasero animals spent more time lying down while Makale cattle spent more time standing during the night. Movement behaviour profiles were used to visually summarise the movement behaviour of cattle in

Petauke District. The profiles showed clear patterns of the three behaviour patterns with noticeable differences between standing and lying. These differences were significant and were highlighted by the score plots which showed Kasero cattle clustered more closely than Makale cattle. The difference in distances between cattle pairs when measured on the score plots were found to be significant for animals in Makale and Kasero. Distances between cattle in Kasero might have clustered more closely on the score plots because they had better well-being than Makale cattle.

CHAPTER 5

Impact of a triple co-administered broad-spectrum treatment on cattle movement behaviour in Petauke District

5.1 Introduction

This chapter looks at the effects of a three drug co-administered treatment directed against tsetse-transmitted (*Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei*.), tick-transmitted (*Theileria parva*, *Anaplasma spp*., *Erlichia ruminantium* and *Babesia spp*.) and pasture-transmitted pathogens of African cattle. Specifically, the chapter looks at the movement behavioural patterns of cattle that were given a treatment when compared with the control group. This was undertaken to try and determine whether instituting a treatment resulted in changes in cattle movement behaviour.

This study was conducted in order to investigate cattle movement behavioural differences of cattle in Petauke District given three co-administered drugs against three commonly occurring diseases and disease types (trypanosomiasis, theileriosis and other tick borne diseases and helminths) in the area. This was done by studying the impact of co-administering the three drug treatment to animals with low circulating haemoglobin levels and observing their movement behaviour patterns (standing, walking and lying down) over a two week period and comparing the results with non-treated control animals. Before the treatment was administered, all animals in the study were monitored for one week.

5.2 Materials and Methods

5.2.1 Study site identification

The study was carried out in Makale Veterinary Camp, Petauke District, in the Eastern Province of Zambia between February and March 2008. Makale was selected as the site for the impact of treatment study based on the results of the

motion sensor study conducted in 2006/07 (Chapter 4). The veterinary camp was purposively selected because it had a higher proportion of animals that had haemoglobin levels below 8 g/dl in the 2006/07 study. This area also had a high proportion of animals that were infected with trypanosomiasis (Chapters 6). The area was also accessible throughout the year as the study was carried out during the rainy season when there were adverse weather conditions.

5.2.1.1 Recruitment of farmers to the study

One to two days before selection of cattle for the study at each village, local cattle owners were requested through the local district veterinary office to present their animals for examination at designated crushpens. The farmers were specifically told that some of their animals would have motion sensors attached and treated against three of the most commonly occurring diseases in the area. At the end of the meeting, farmers were requested to present their animals for clinical examinations at the designated crushpens. This was in addition to the sensitization carried out at the beginning of the Programme (Chapter 2, section 2.1.1.). Cattle were presented for clinical examination and/or attachment of motion sensors at the designated crushpen by their owners or herdsmen.

5.2.2 Study design

5.2.2.1 Cattle selection and allocation to treatment groups

Cattle owners or their herdsmen presented a total of 204 cattle at the pre-selection stage in Makale for screening by clinical examination and blood sampling. Some cattle farmers that presented their animals for the current study also participated in the 2006/07 study. Using a Hemocue Hb 201+ haemoglobinometer (HemoCue AB, Ängelholm, Sweden), haemoglobin values

were measured and recorded for each animal as detailed in Chapter 2 (Section 2.1.4.1). All animals were fitted with numbered ear tags for identification.

A range of haemoglobin values were obtained from the initial pre-selection screening of the experiment and used to select 40 animals for the next stage of the study. The selected 40 animals were paired and then randomly allocated to either the treatment or control group. Later one motion sensor was attached to each animal in each group. The 40 animals were selected and matched based on the following criteria;

- All animals had haemoglobin levels below 8 g/dl
- All animals in the pairs were as close as possible the same age
- All animals in the pairs were the same sex
- All animals in the pairs were co-grazing and belonging to the same kraal
- Draft animals were excluded from the study
- Animal pairs had as close as possible matching haemoglobin levels at the pre-selection stage

After the selection of the 20 co-grazing pairs, the owners were informed about the next stage of the study and asked to present their animals for motion sensor attachment.

5.2.2.2 Sample collection and duration of study

Table 5. 1 Timeline of impact of treatment on movement behaviour study

Day	***Procedure***
Day -9 Pre-selection	Cattle screened for haemoglobin in Makale (n = 204). Haemoglobin recorded.
-7	Motion sensors attached to twenty co-grazing cattle pairs. Haemoglobin recorded.
0	Motion sensor data downloaded. Haemoglobin recorded. Drugs administered to one animal in each pair. The other pair becomes control.
7	Motion sensor data downloaded. Haemoglobin recorded.
14	Final motion sensor data downloaded. Removal of motion sensors from animals. Haemoglobin recorded.

Motion sensor attachment was carried out as detailed in Chapter 2 (Section 2.1.5). On the day of motion sensor attachment, haemoglobin values were measured and recorded. This was repeated on days zero, seven and fourteen of the experiment (Table 5. 1). On days zero and seven, motion sensor data was also downloaded to a laptop computer (Chapter 2, section 2.1.7) to avoid losing all data in the event of loss of a motion sensor unit. All animals in the co-grazing pairs had five haemoglobin and clinical parameter readings in addition to the three weeks' worth of motion sensor data. In week one, one animal was excluded from the study because the motion sensor fell off during the period. Data from this animal's motion sensor during weeks two and three was included in the analyses. After one week of motion sensor attachment, one animal in the co-

grazing pair was selected and had a treatment administered. The other animal in the co-grazing pair was not treated and acted as the control.

5.2.3 Data recording and storage

Clinical data was recorded on to the cattle data collection forms (Chapter 4, table 4.2) and later transferred to a Microsoft Excel (2003) workbook for storage and analyses. Motion sensor data was downloaded in the field onto a laptop computer via a USB cable using IceTag™ Analyser software (Research Version 2.003) and saved as comma separated variable (.csv) files before being exported to Microsoft Excel (2003) for storage. Data analyses were carried out as described in Chapter 4 (Section 4.2.4)

5.2.4 Triple co-administered broad-spectrum drug treatment

The treatment consisted of using veterinary drugs that are commonly used to treat these diseases in the study area and readily available at the Petauke District Veterinary Office or local agriculture input shops (Mubanga, 2009). The three most common disease problems known in the study site are trypanosomiasis, theileriosis and pasture-transmitted diseases (Mubanga, 2009; Nambota et al., 1994; Sinyangwe et al., 2004). The drugs used to treat against these pathogens were diminazene aceturate (Berenil® - Intervet), long-acting Oxytetracycline (Oxyject LA® — Dopharma, Netherlands) and Albendazole (Dopharma, Netherlands). Owners of all animals in the treated and control groups were asked not administer any drugs to their cattle during the study period.

5.2.4.1 Trypanocidal Chemotherapy

The trypanocidal drug that is used was diminazene aceturate (Berenil®) at a dose rate of 3.5 mg/kg body weight by the intramuscular route. This was achieved by diluting a 2.36 g packet of the Berenil granules into 12.5 ml of sterile water, and administering 1 ml/20 kg body weight. This drug was also considered to be effective against *Babesia spp*. This was a once only dose that was given on day zero of the study.

5.2.4.1 Tick-borne Disease Chemotherapy

The drug used that is effective against theileriosis, anaplasmosis and cowdriosis was long acting Oxytetracycline (Oxyject LA® — Dopharma, Netherlands) at 20 mg/kg by the intramuscular route. This is the drug that is readily available at the Petauke District Veterinary Office and is commonly used in the treatment of tickborne diseases (Mubanga, 2009). Tetracyclines can be used to treat animals with East Coast fever and other tick borne diseases, but as with the other drugs, treatment must be initiated early at the onset of clinical signs (Merck Veterinary Manual, 2006; Siegel et al., 2006; Young et al., 1988). This was a once only dose that was given on day zero of the study.

5.2.4.3 Pasture-transmitted Helminths Chemotherapy

The drug used that is effective against the pasture-transmitted helminths was the broad spectrum anthelmintic Albendazole (Dopharma, Netherlands**)** at 10 mg/kg body weight and was administered orally as a drench using a drench gun. This was also a once only dose that was given on day zero of the study.

5.3 Results

The results in this study consisted of the motion sensor data output and haemoglobin readings observed and recorded during the pre-selection stage, on days minus seven (-7), day zero (0), day seven (7) and day fourteen (14). The results were analysed in two stages. The first stage consisted of pre-treatment results that were obtained on pre-selection day, day minus seven and on day zero. Blood samples on the day of treatment (day zero) were collected prior to drug administration. Secondly, post-treatment results were obtained on days seven and fourteen following the co-administration of the three drugs.

5.3.1 Haemoglobin values of cattle in the treated and control groups

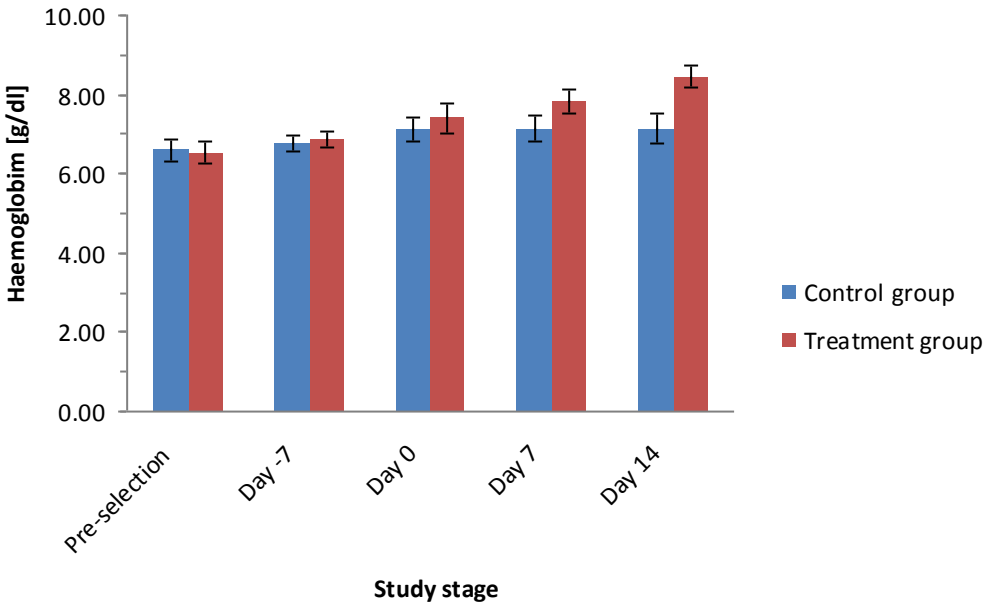

Figure 5. 1 Mean haemoglobin levels for cattle in the control and treated groups that had motion sensors attached in Makale Veterinary Camp. Error bars represent standard error for the data

The mean haemoglobin values for cattle during the impact of treatment study in Makale Veterinary Camp are shown in Figure 5. 1. The mean haemoglobin value

for control cattle during the pre-treatment stage (pre-selection, day -7 and day 0) was 6.87 g/dl, while that for cattle in the treated group (pre-selection, day -7 and day 0) was 6.94 g/dl. During the pre-treatment stage, there was no significant difference between the mean haemoglobin values of control (6.87 g/dl) and treated cattle (6.94 g/dl) in Makale (paired t-test; difference of means = -0.06, 95% CI [-0.3, 0.2], p = 0.63).

The mean haemoglobin value for control cattle during the post-treatment stage (days 7 and 14) was 7.14 g/dl, while that for cattle in the treated group was 8.14 g/dl. There was a highly significant difference between the mean haemoglobin values of the control (7.14 g/dl) and treated cattle groups (8.14 g/dl) at the post-treatment stage (paired t-test; difference of means = -1, 95% CI [-1.5, -0.5], $p < 0.001$).

There was no significant difference between the mean haemoglobin values of cattle in the control group at the pre-treatment stage (6.87 g/dl) and post-treatment (7.14 g/dl) stage (paired t-test; difference of means = -0.3, 95% CI [-0.7, 0.1], p = 0.13). There was a highly significant difference between cattle in the treated group at the pre-treatment stage (6.94 g/dl) and post-treatment stage (8.14 g/dl) (paired t-test; difference of means = -1.2, 95% CI [-1.6, -0.85], $p < 0.001$).

5.3.2 Step counts of cattle

Figure 5. 2 shows the mean steps per day of 19 pairs of co-grazing cattle during the week before they were treated. One pair was not included in the analysis because one motion sensor from the pair dropped in the middle of the experiment. The mean number of steps per day for the control group of cattle

was 6,854 steps while the treated group had a mean step count per day of 6,697. During the one week before treatment, there was no significant difference between the mean number of steps of the control group of animals and those in the treated group (paired t-test; diffference of means = -157, 95% CI [-1032, 717], p = 0.71). There were 8 co-grazing cattle pairs with animals in the treated group having significantly more mean number of steps per day during the week before treatment. There were 7 co-grazing cattle pairs with animals in the treated group having significantly fewer mean number of steps during the same period. There were 4 co-grazing cattle pairs that did not show any significant differences between the treated and control animals' mean number of steps per day during the week before treatment.

Figure 5. 3 shows the mean number of steps taken by 20 pairs of co-grazing cattle during the week after treatment. The mean number of steps per day taken during this week by the control group of cattle was 7,191 steps while the treated group of cattle had a mean number of steps of 6,369 per day. This difference in the number of steps taken between the control and treated groups during the first week after treatment was found to be significant (paired t-test; diffference of means = -823, 95% CI [-1502, -143], $p < 0.05$). There were 2 co-grazing cattle pairs in the treated group with animals having significantly more mean number of steps per day during the week after treatment. There were 10 co-grazing cattle pairs in the treated group with animals having significantly fewer mean number of steps during the same period. There were 8 co-grazing cattle pairs that did not show any significant differences between the treated and control animals' mean number of steps per day during the week after treatment.

During the second week after treatment (Figure 5. 4), the control group had 7,116 steps while treated group had a mean number of steps per day of 8,131 steps. The treated cattle group had significantly more steps per day than the control group (paired t-test; diffference of means = -1014, 95% CI [3,2026], $p < 0.05$). There were 7 co-grazing cattle pairs with animals in the treated group having significantly more steps per day during the second week after treatment. There were 2 co-grazing cattle pairs with animals in the treated group having significantly fewer mean number of steps during the same period. There were 11 co-grazing cattle pairs that did not show any significant differences between the treated and control animals' mean number of steps per day during the second week after treatment.

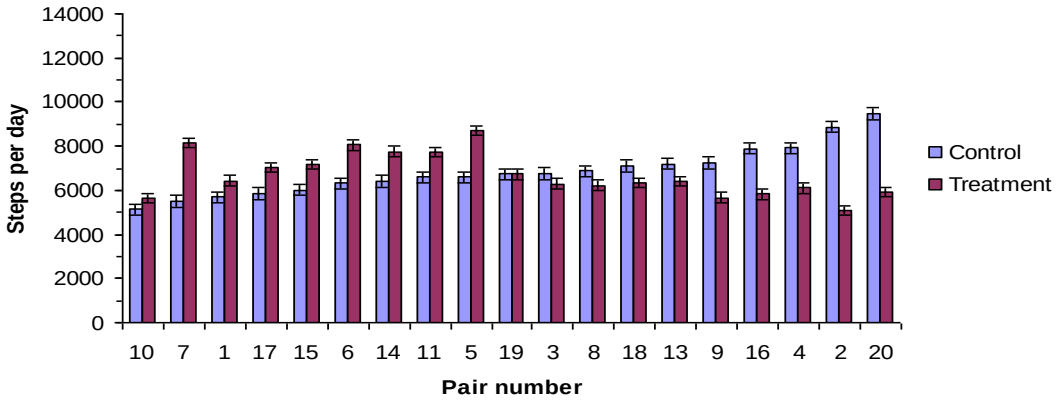

Figure 5. 2 Mean number of steps taken per day by cattle co-grazing in pairs over the week prior to treatment in Makale. Cattle pair numbers are shown. Error bars represent standard error for the data.

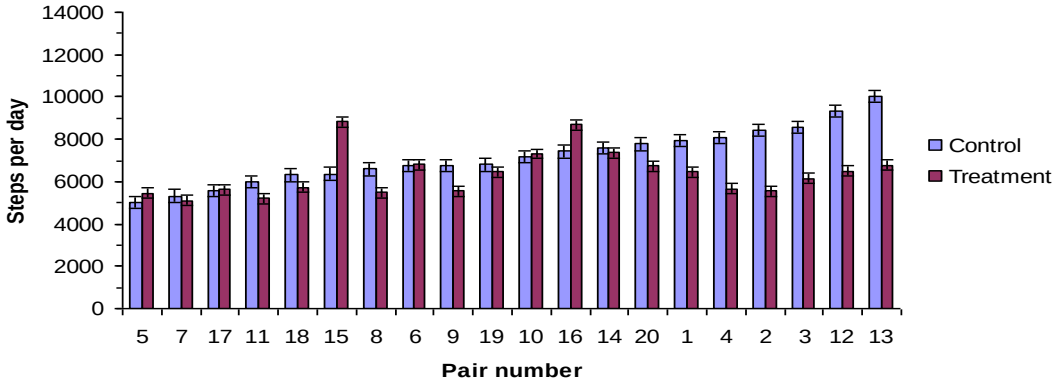

Figure 5. 3 Mean number of steps taken per day by cattle co-grazing in pairs in the first week after treatment in Makale. Cattle pair numbers are shown. Error bars represent standard error for the data.

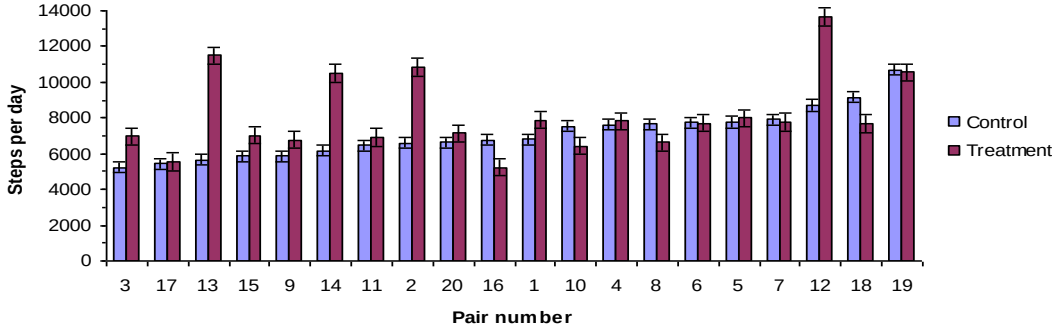

Figure 5. 4 Mean number of steps taken per day by cattle co-grazing in pairs in the second week after treatment in Makale. Cattle pair numbers are shown. Error bars represent standard error for the data.

There was no significant difference between treated cattle one week before treatment (6,697 steps per day) and one week after treatment (6,369 steps per day), (paired t-test; diffference of means = -336, 95% CI [-413, 1084], p = 0.36), (Table 5. 2). Cattle in the treated group took significantly more steps during the second week after treatment (8,131 steps per day) than they did during the week before treatment (paired t-test; diffference of means = 1,142, 95% CI [-2100, -184], $p < 0.05$).

There was no significant difference between cattle in the control group one week before treatment (6,854 steps per day) and one week after treatment (7,191 steps per day) (paired t-test; diffference of means = 224, 95% CI [-831, 382], p = 0.45). There was also no significant difference between cattle in the control group one week before treatment and during the second week after treatment (7,116 steps per day) (paired t-test; diffference of means = 178, 95% CI [-1038, 683], p = 0.67). Figure 5. 5 shows mean the number of steps per day and the step differences between cattle in the treated and control groups before and after treatment in Makale.

The percentage difference in the mean number of steps per day between the treated group and control group during the one week before treatment was -2.3%. During the first week after treatment, the percentage difference in the mean number of steps per day between the two groups was -12.9% while it was 12.5% during week two. The percentage change in the mean number of steps per day from the week before treatment to one week after treatment for treated cattle was -5.0%. The percentage change in the mean number of steps per day from the week before treatment to the second week after treatment for treated cattle was 17.0%.

The percentage change in the mean number of steps per day from the week before treatment to one week after treatment for control cattle was 3.3%. The percentage change in the mean number of steps per day from the week before treatment to two weeks after treatment for control cattle was 2.6% (Table 5. 3).

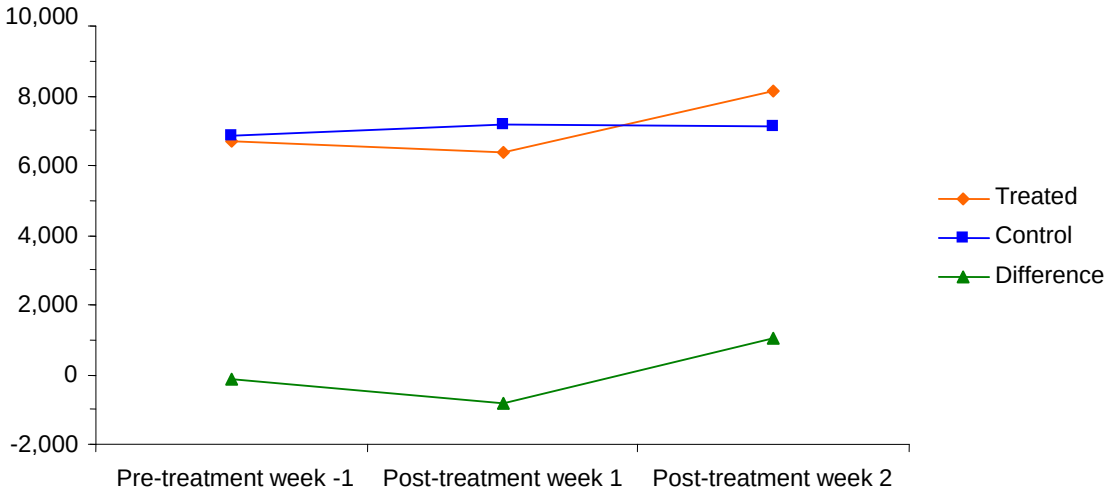

Figure 5. 5 Mean number of steps per day and the step differences between treated and control cattle groups before and after treatment in Makale

Table 5. 2 Mean number of steps taken by cattle per day in the week before and two weeks following treatment in Makale

	Week 1 pre-treatment			Week 1 post-treatment			Week 2 post-treatment		
Pair Number	*Treatment*	*Control*	*Difference*	*Treatment*	*Control*	*Difference*	*Treatment*	*Control*	*Difference*
1	6430	5694	736	6461	7933	-1472	7886	6794	1,092
2	5096	8872	-3776	5546	8440	-2894	10850	6592	4,258
3	6296	6758	-462	6146	8583	-2437	6974	5248	1,726
4	6106	7930	-1824	5655	8061	-2406	7827	7626	201
5	8699	6594	2105	5465	5035	430	8006	7764	242
6	8061	6321	1740	6806	6738	68	7702	7753	-51
7	8134	5507	2628	5118	5319	-201	7780	7928	-148
8	6223	6885	-662	5475	6588	-1113	6625	7668	-1,043
9	5673	7238	-1565	5544	6753	-1209	6775	5865	910
10	5627	5144	484	7310	7184	126	6432	7520	-1,088
11	7746	6588	1158	5212	5988	-776	6925	6463	461
12	*	*	*	6504	9332	-2828	13665	8711	4,954
13	6416	7201	-785	6765	10029	-3265	11496	5667	5,830
14	7752	6407	1344	7355	7610	-256	10500	6157	4,343
15	7159	6025	1135	8813	6369	2445	7020	5849	1,171
16	5822	7889	-2067	8673	7426	1248	5232	6774	-1,542
17	7024	5858	1166	5617	5564	53	5548	5445	103
18	6322	7111	-789	5726	6315	-590	7680	9167	-1,487
19	6729	6736	-7	6452	6792	-340	10559	10691	-133
20	5931	9476	-3545	6730	7767	-1037	7129	6643	486
	6,697	6,854	-157	6,369	7,191	-823	8,131	7,116	1,014

* = data missing due to motion sensor falling off the animal

Table 5. 3 Paired t-tests for differences in mean number of steps taken daily by cattle in Makale before and after treatment.

	Treated Group	*Control Group*	*Difference*	*Percentage difference*	**p value**
One week before treatment					
Mean	6,697	6,854	-157 [-1032, 717]	-2.3%	0.71
One week after treatment					
Mean	6, 369	7, 1 91	-823 [-1502, -143]	-12.9%	< 0.05*
Difference from pre-treatment	-336 [-413, 1084]	224 [-831, 382]			
(Percentage change)	(-5.0%)	(3.3%)			
p value	0.36	0.45			
Two weeks after treatment					
Mean	8,131	7, 116	1014 [3,2026]	12.5%	< 0.05*
Difference from pre-treatment	1,142 [-2100, -184]	178 [-1038, 683]			
(Percentage change)	(17%)	(2.6%)			
p value	< 0.05*	0.67			

Figures in parentheses are 95% confidence intervals, * = significant

Figure 5. 6 shows the mean number of steps taken per day by cattle in Makale in the treated and control groups in the week before and two weeks following treatment. The figure shows that in the week before treatment, cattle in the treated and control groups had between 5,000 and slightly over 9,000 steps per day. From one week to two weeks after treatment, the mean number of steps per day for five cattle in treated group increased to over 10,000 steps per day while only one animal in the control group reached this high number of steps.

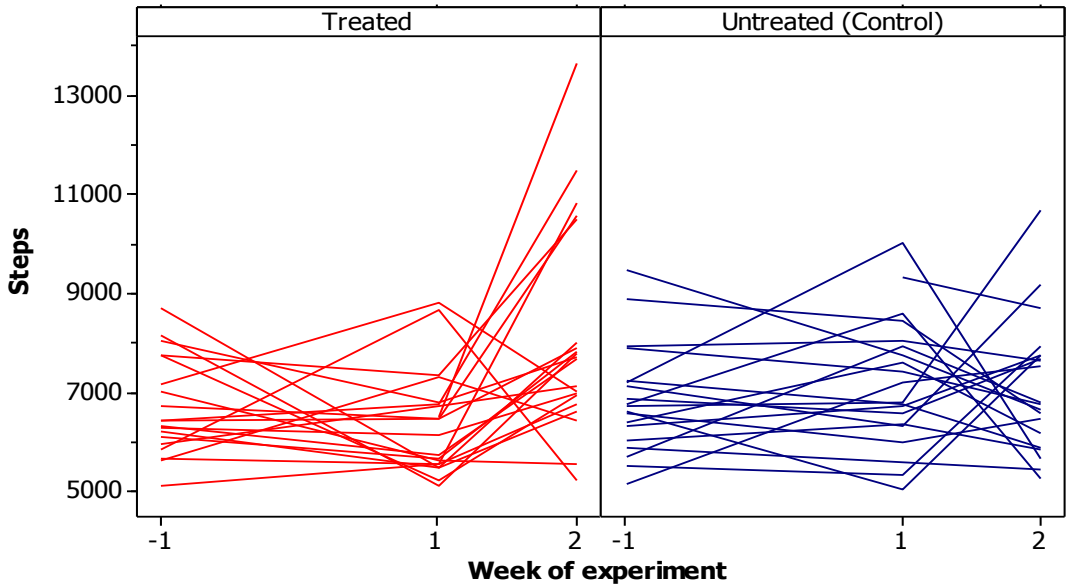

Figure 5. 6 Mean number of steps taken per day by cattle in Makale in the treated and control groups in the week before and two weeks following treatment.

Figure 5. 7 shows the mean number of steps taken per day by individual cattle co-grazing in pairs in the week before and two weeks following treatment in Makale. The figure shows that one week before treatment, a total of 9 animals in the treated group had more mean number of steps per day than control cattle. In week 1 of the experiment, 6 treated cattle took more steps than their control counterparts. In week 2 of the experiment, 13 treated cattle took more steps than cattle in the control group.

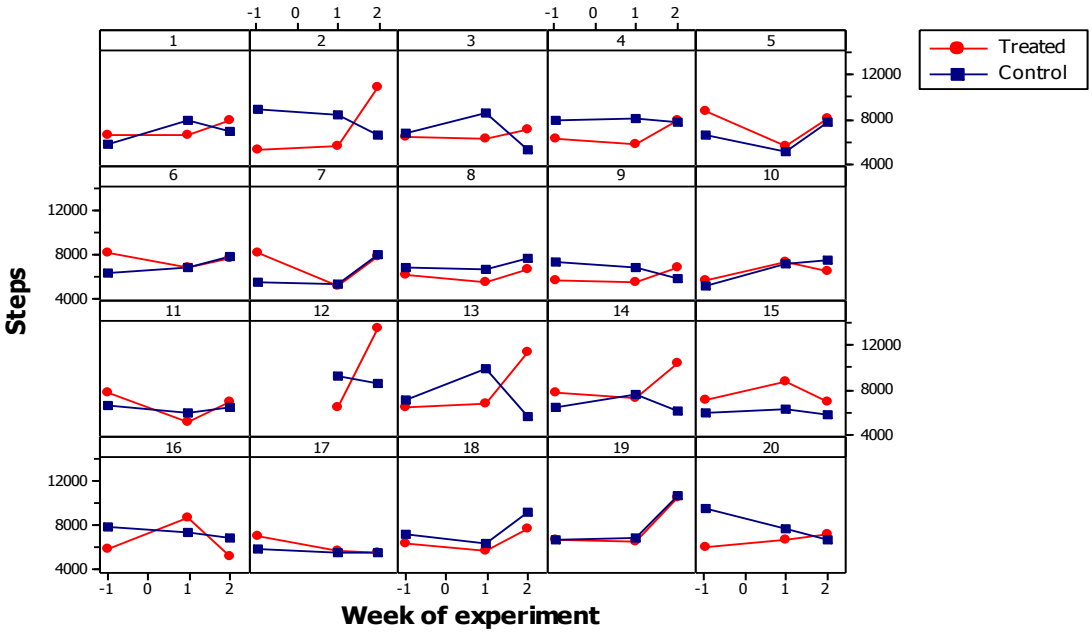

Figure 5. 7 Mean number of steps taken per day by individual cattle co-grazing in pairs in the week before and two weeks following treatment in Makale.

5.3.3 Cattle behaviour profiles

Cattle behaviour profiles were created for the animals in the study. The behaviour profiles were mean representations of group cattle behaviour in each hour over the specified experimental period. The group profiles of all the animals in the pre-treatment and post-treatment stages were also compared for any differences in their behaviour.

5.3.3.1 Cattle standing behaviour profiles – post-treatment

Boxplots were employed to visualize the mean group behaviour of the cattle in the pre-treatment and post-treatment stages of the experiment. Boxplots for the pre-treatment group are presented in Appendix 3**.** Figure 5. 8 is a boxplot of the mean standing behaviour of the 20 treated and 20 control cattle in Makale during

the post-treatment stage. The figure shows that, on average there is some standing behaviour in any given hour over a 24 hour period in both the treated group and controls. Between the hours of 20:00 in the evening and 04:00 hours in the morning, animals spend around 20% of their time standing. Between 04:00 hours in the morning and 06:00 hours in the morning, there is a sharp increase in standing behaviour in both groups. Standing behaviour then remains almost constant until 17:00 hours when it starts to decline apart from a small reduction around mid-morning. This behaviour is observed in both the treated group and the controls.

5.3.3.2 Cattle walking behaviour profiles – post-treatment

Figure 5. 9 shows a boxplot of the mean walking behaviour of the 20 treated and 20 control cattle in Makale during the post-treatment stage. The figure shows that there is none or very little walking between 19:00 hours in the evening and 04:00 hours in the morning. Cattle seem to start the day at the same time (05:00 hours) in the two groups. From 05:00 hours there is a sharp increase in walking behaviour until around 08:00 hours. From 09:00 hours walking behaviour starts to decline reaching a low at 11:00 hours. Over the midday period between 12:00 and 13:00, walking behaviour is low but rises from 14:00 hours and peaks at 16:00 hours before starting to decline. The walking behaviour shows two peaks, one in the morning and one in the afternoon. This behaviour was similar in both the treated group and the controls.

5.3.3.3 Cattle lying behaviour profiles – post-treatment

Mean lying behaviour for the treated and control groups in Makale is shown in Figure 5. 10. The two profiles show that lying behaviour is mainly restricted to between 17:00 hours in the evening and 04:00 hours in the morning. There is also some lying behaviour between the hours of 10:00 hours and 13:00 hours.

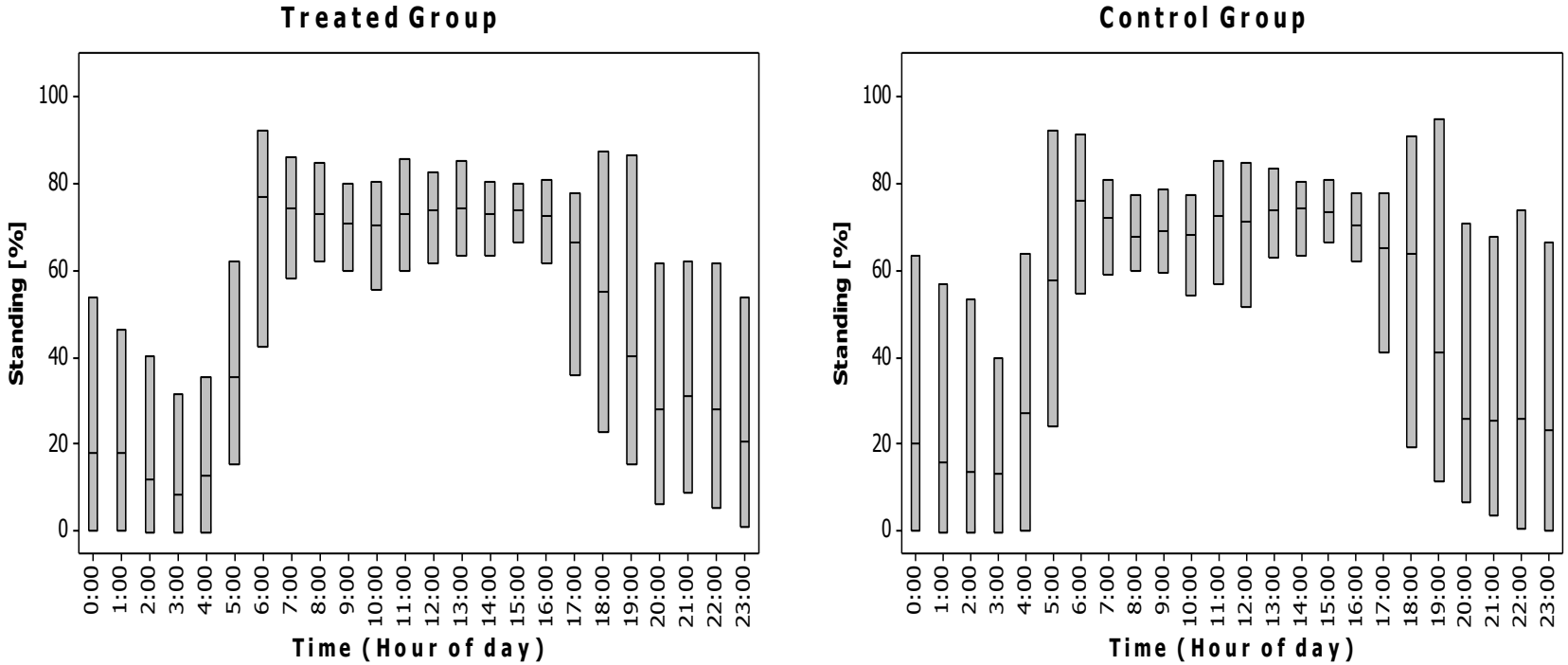

Figure 5. 8 Post-treatment standing behaviour profiles as a mean percentage of time cattle spent standing in each hour of the day in Makale during the two week period. Each box indicates the lower and upper quartiles representing the points below which 25 % and 75 % of the observations lie respectively. The median value is shown within each box as a horizontal line. This is the value below which half, and above which half, the observations lie.

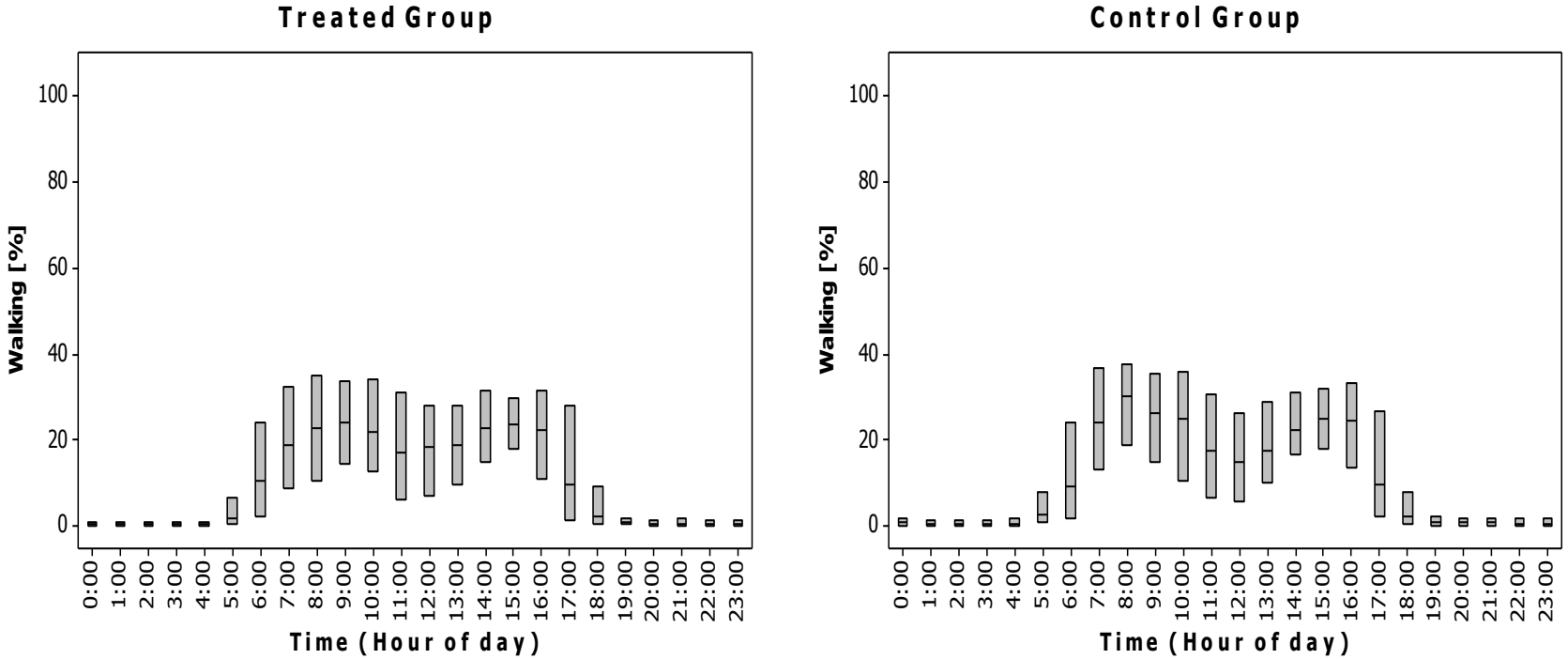

Figure 5. 9 Post-treatment walking behaviour profiles as a mean percentage of time cattle spent walking in each hour of the day in Makale during the two week period.

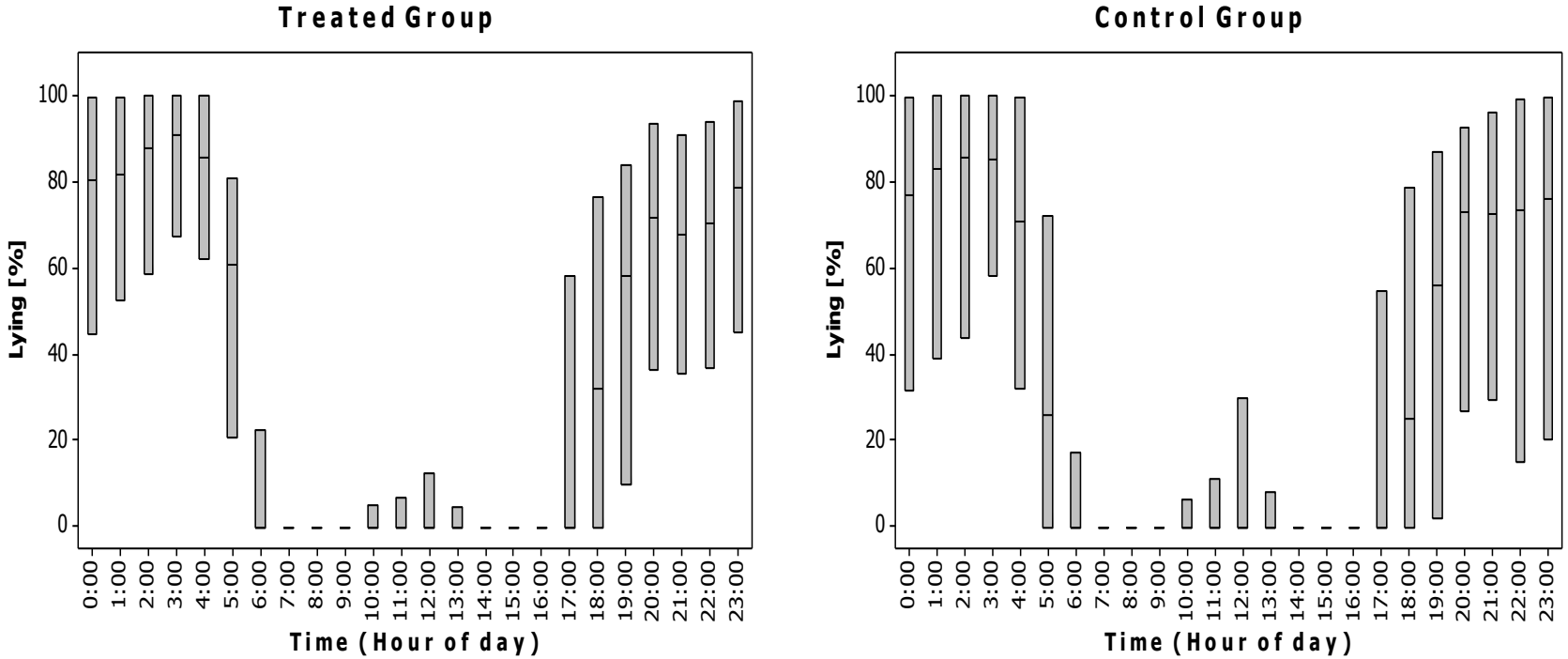

Figure 5. 10 Post-treatment lying behaviour profiles as a percentage of time cattle spent lying down in each hour of the day in Makale during the two week period.

5.3.3.4 Mean cattle time budgets for treated and control cattle

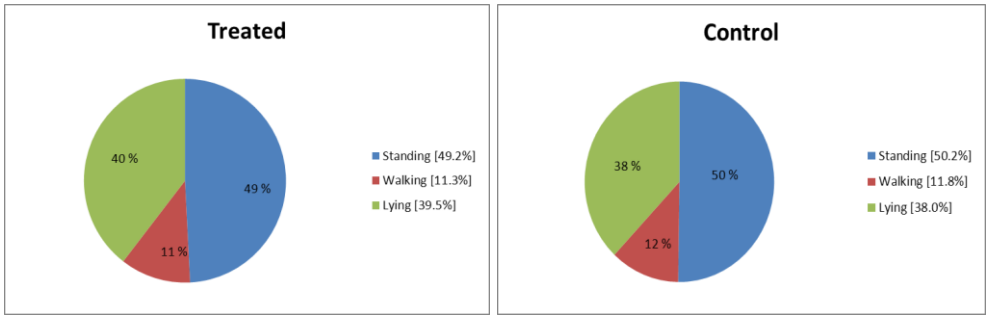

Figure 5. 11 Time budgets for cattle in the treated and control groups during the pre-treatment stage of the study. Pie charts show the mean time cattle spent standing, walking and lying down during the one week prior to treatment.

The mean pre-treatment time budgets (percentage of time cattle spent standing, walking or lying down) for cattle in the control and treated groups of the study are shown in Figure 5. 11. Control cattle spent a total of 50% of their time standing while 38% of their time was spent lying down and 12% walking. Cattle in the treated group spent 49% of their time standing, 40% lying down and 11% walking. The difference in mean time spent standing for cattle in the control and treated groups during the pre-treatment stage was found not to be significant (paired t-test; mean difference = 0.99, 95 % CI [-0.4, 2.4], p = 0.17). There was a small but significant difference between the mean time spent lying down by cattle in the control and treated groups (paired t-test; mean difference = -1.64, 95 % CI [-3.3, -0.02], p = 0.047) with treated animals lying down more. There was also a small but significant difference between the mean time spent walking by cattle in the two groups (paired t-test; mean difference = 0.67, 95 % CI [0.06, 1.3], p = 0.03).

The mean post-treatment time budgets during weeks one and two for cattle in the control and treated groups of the study are shown in Figure 5. 12. Control

cattle one week after treatment spent a total of 46.7% of their time standing while 41.3% of their time was spent lying down and 11.9% walking. Cattle in the treated group during week one spent 47.8% of their time standing, 41.7% lying down and 10.5% walking.

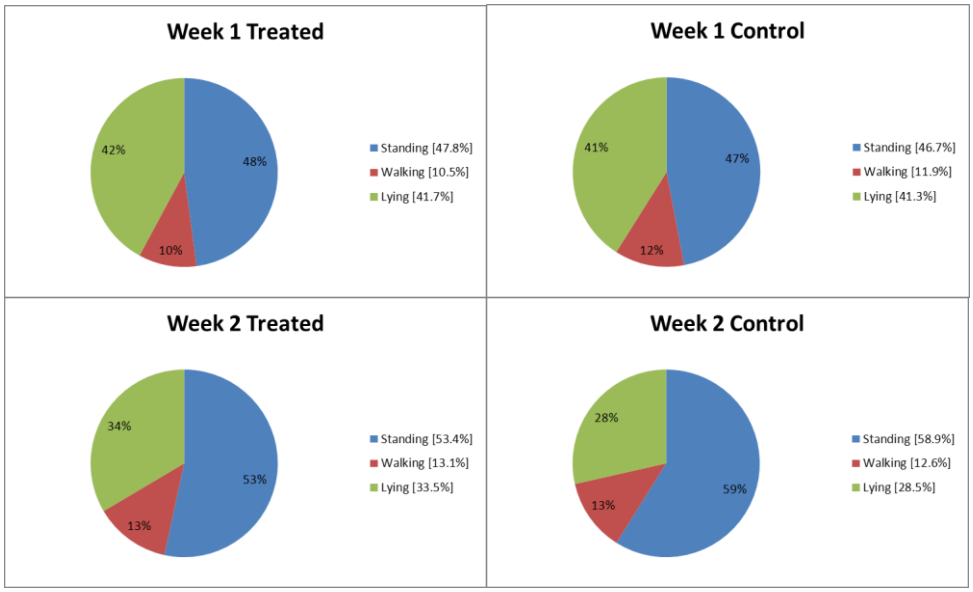

Figure 5. 12 Time budgets for cattle in the control and treated groups during weeks one and two of the post-treatment stage of the study. Pie charts show the mean time cattle spent standing, walking and lying down during period.

The difference in mean time spent standing for cattle in the control and treated groups during the first week of the post-treatment stage was found not to be significant (paired t-test; mean difference = -1.0, 95 % CI [-2.4, 0.4], p = 0.16). There was also no significant difference between the mean time spent lying down by cattle in the control and treated groups (paired t-test; mean difference = -0.4, 95 % CI [-2.1, 1.2] p = 0.62). There was a highly significant difference between the mean time spent walking by cattle in the two groups with control cattle walking more (paired t-test; mean difference = 1.4, 95 % CI [0.79, 2.1], p < 0.001). Control cattle two weeks after treatment spent a total of 58.9% of their time standing while 28.5% of their time was spent lying down and 12.6%

walking. Cattle in the treated group during week two spent 53.4% of their time standing, 33.5% lying down and 13.1% walking.

The difference in mean time spent standing for cattle in the control and treated groups during the second week of the post-treatment stage was found to be highly significant with control animals standing more (paired t-test; mean difference = 5.4, 95 % CI [4.0, 6.9], $p < 0.001$). There was also a highly significant difference between the mean time spent lying down by cattle in the control and treated groups with treated animals lying down more (paired t-test; mean difference = -5.0, 95 % CI [-6.5, -3.5] $p < 0.001$). There was a small but significant difference between the mean time spent walking by cattle in the two groups with treated animals walking more (paired t-test; mean difference = -0.6, 95 % CI [-1.1, -0.002], $p < 0.05$).

5.3.4 Body condition score of cattle in treatment study

The mean body condition score of all cattle in Makale during the treatment study was 4.4. Cattle in the treated group had a mean body condition score of 4.2 while the score for control cattle was 4.6. There was no significant difference between the condition scores of treated and control cattle in Makale (paired t-test; mean difference = -0.45, 95 % CI [-1.0, 0.12], $p = 0.12$).

5.3.5 Principal components analysis (PCA)

A principal components analysis (Chapter 4) was used to further analyse the data. The PCA was used to reduce the multidimensional data set to lower dimensions and used to identify new, meaningful, hidden differences between the data.

5.3.5.1 PCA before treatment

The principal components analysis for studying the pre-treatment cattle motion sensor data was carried out for the one week that motion sensors were attached on 39 animals in the study before the treatment was carried out. Data from one animal was excluded because the motion sensor fell off during the first week of attachment. The PCA data output gives the coefficients (or loadings) of each principal component; the values of each individual on each component (known as the scores on each component) and the variances of the scores for each component (known as the eigenvalues). Complete loadings for PCA results in this chapter are shown in appendix 2. The output is arranged so that the component with the largest variance (eigenvalue) comes first; the one with the next largest variance comes second, and so on. Table 5. 4 shows the eigenvalues of the first 18 principal components with a cumulative contribution of 95% of overall variability.

Table 5. 4 Principal component eigenvalues and proportions

PC	1	2	3	4	5	6	7	8	9
Eigenvalue	19.577	10.102	7.805	5.143	4.285	3.321	3.044	2.603	2.364
Proportion	0.272	0.140	0.108	0.071	0.060	0.046	0.042	0.036	0.033
Cumulative	0.272	0.412	0.521	0.592	0.652	0.698	0.740	0.776	0.809

PC	10	11	12	13	14	15	16	17	18
Eigenvalue	2.022	1.503	1.321	1.178	0.997	0.916	0.802	0.753	0.630
Proportion	0.028	0.021	0.018	0.016	0.014	0.013	0.011	0.010	0.009
Cumulative	0.837	0.858	0.876	0.893	0.906	0.919	0.930	0.941	0.950

The first thirteen principal components had eigenvalues above one and had cumulative eigenvalue proportion of 0.893. The original 72 dimensional data was therefore reduced to thirteen accounting for 89.3% of the variability of the data. The first four principal components together accounted for 59.2% of the overall variability in the data (Table 5. 4). This shows that only four of the principal components were able to account for more than half of the variability in the data. The first principal component was the major contributor and accounted for 27.2 % of the overall variability in the data. Principal components two, three and four accounted for 14.0 %, 10.8 % and 7.1 % respectively.

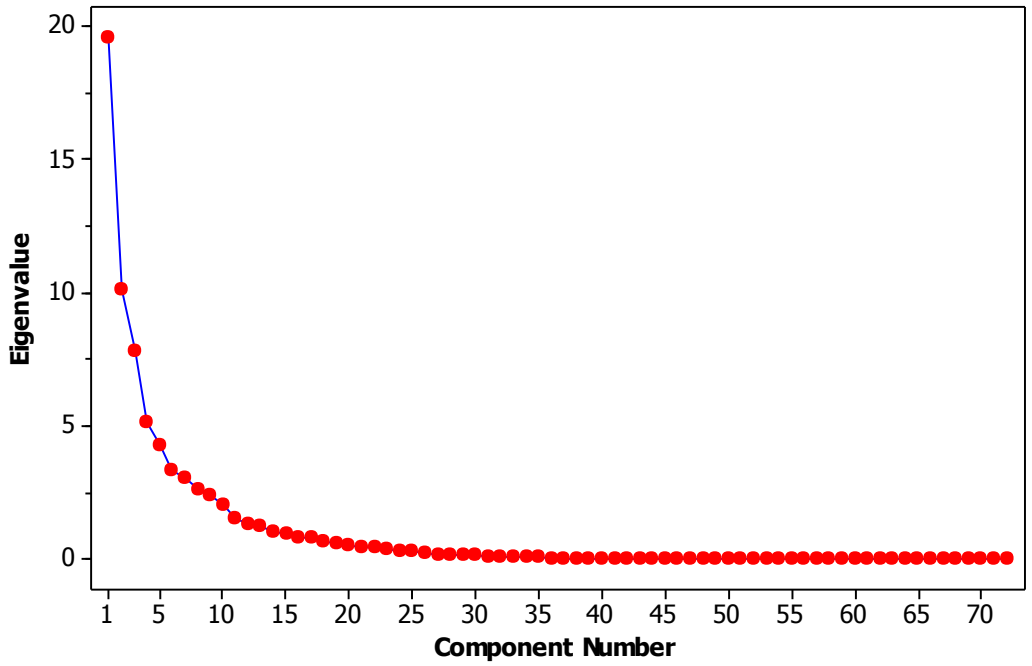

Figure 5. 13 Scree plot based on 72 variables expressing cattle behaviour before treatment in Makale

The results of the PCA based on 72 variables expressing cattle behaviour at the pre-treatment stage are represented by a scree plot (Figure 5. 13). The scree plot (Chapter 4, section 4.4.4) is a plot of the eigenvalue associated with a principal component versus the number of the component. The scree plot is used to judge the relative magnitude of the eigenvalues. The cut off point for factor extraction is placed at the "elbow" of the graph or at a point where the eigenvalue is above one. The scree plot shown in Figure 5. 13 was generated from the pre-treatment motion sensor data expressing the three movement behaviour variables. The plot shows that the first four principal components have eigenvalues above five and are larger than the other principle components which have smaller and closer matching eigenvalues. Together, the first four principal components represent 59.2% of the total variability. Thus, most of the data structure can be captured in four underlying dimensions. The remaining principal components account for a

smaller proportion of the variability. The eigenvalue (scree) plot provides this information visually.

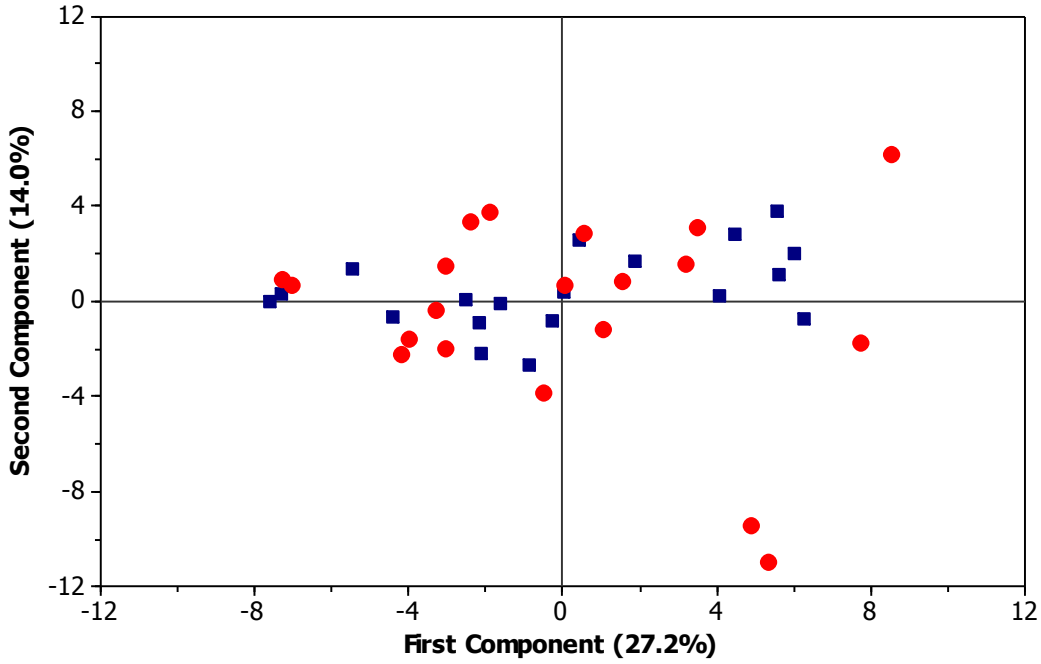

Figure 5. 14 Score Plot of the first and second Principal components resulting from the PCA of pre-treatment motion sensor data of cattle in the treated group (■) and the control group (●)over a period of one week in Makale.

The results of the PCA were also represented by two-dimensional plots called the score plots (Figure 5. 14 and Figure 5. 15). The score plots were based on the PCA and showed similarities and differences among the cases in two-dimensional space. The score plot diagrams were made by plotting the scores of the first and second (Figure 5. 14) and second and third (Figure 5. 15) principal components. Principal components plotted in Figure 5. 14 and Figure 5. 15 represents a combined contribution to overall variability of 41.2% and 33.2% respectively. Score plots highlight cases with similar patterns because they will cluster more closely within the plot. Score plots were generated from the PCA to determine the animal's relative positions in two dimensional space.

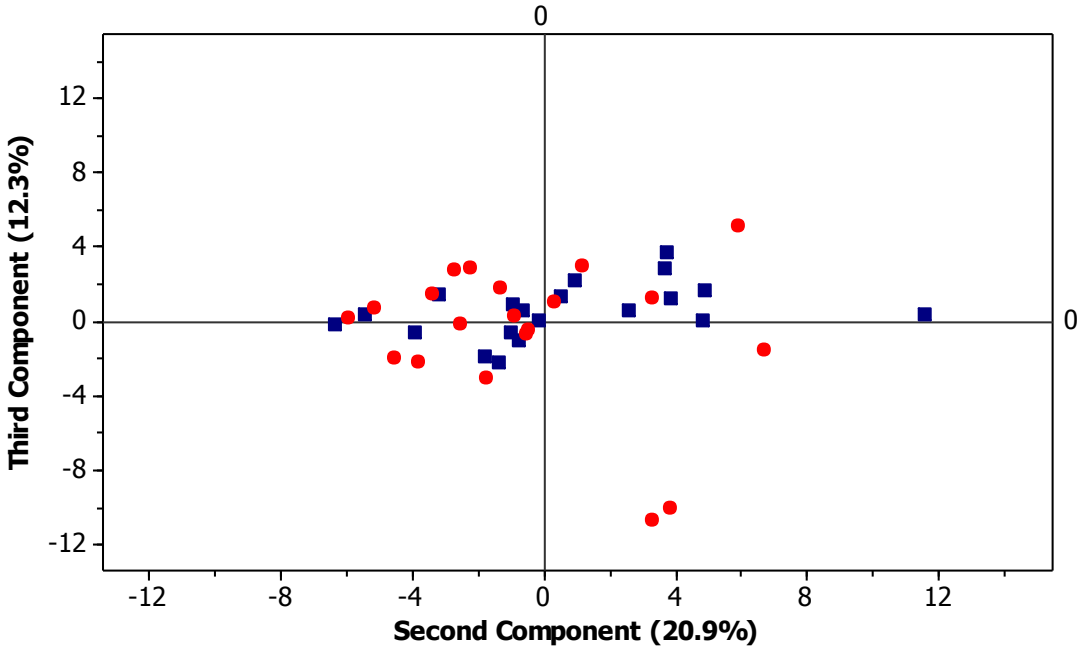

Figure 5. 15 Score Plot of the Second and Third Principal components resulting from the PCA of pre-treatment motion sensor data of cattle in the treated group (■) and the control group (●) over a period of one week in Makale.

The positions in two dimensional space of the treated groups and control groups as indicated in the score plots (Figure 5. 14, Figure 5. 15) shows that the majority of the cases from the two groups were evenly distributed and formed no distinct clusters. This indicated that the animals in the treated group and the control group did not have any major differences between them during the one week that motion sensors were attached before treatment.

5.3.5.2 PCA after treatment

The principal components analysis for studying the post-treatment cattle motion sensor data was carried out for the two weeks that motion sensors were attached on all of the 40 animals in the study after the treatment was carried out. Table 5. 5

shows the eigenvalues of the first 18 principal components with a cumulative contribution of 96.2% of overall variability.

Table 5. 5 Principal component eigenvalues and proportions

PC	1	2	3	4	5	6	7	8	9
Eigenvalue	22.841	10.232	7.321	5.829	3.859	3.629	2.882	2.676	2.036
Proportion	0.317	0.142	0.102	0.081	0.054	0.050	0.040	0.037	0.028
Cumulative	0.317	0.459	0.561	0.642	0.696	0.746	0.786	0.823	0.851

PC	10	11	12	13	14	15	16	17	18
Eigenvalue	1.561	1.289	1.195	0.909	0.867	0.689	0.570	0.501	0.411
Proportion	0.022	0.018	0.017	0.013	0.012	0.010	0.008	0.007	0.006
Cumulative	0.873	0.891	0.908	0.920	0.932	0.942	0.950	0.957	0.962

The first twelve principal components had eigenvalues above one and had cumulative eigenvalue proportion of 0.908. The original 72 dimensional data was therefore reduced to twelve accounting for 90.8% of the variability of the data. The post-treatment PCA yielded a four factor structure accounting for 64.2% of the total variance of the data (Table 5. 5). Principal component one contributed the greatest amount of variability with 31.7% while PCs two, three, four, five and six contributed 14.2%, 10.2%, 8.1%, 5.4% and 5.0% respectively to the overall data variability.

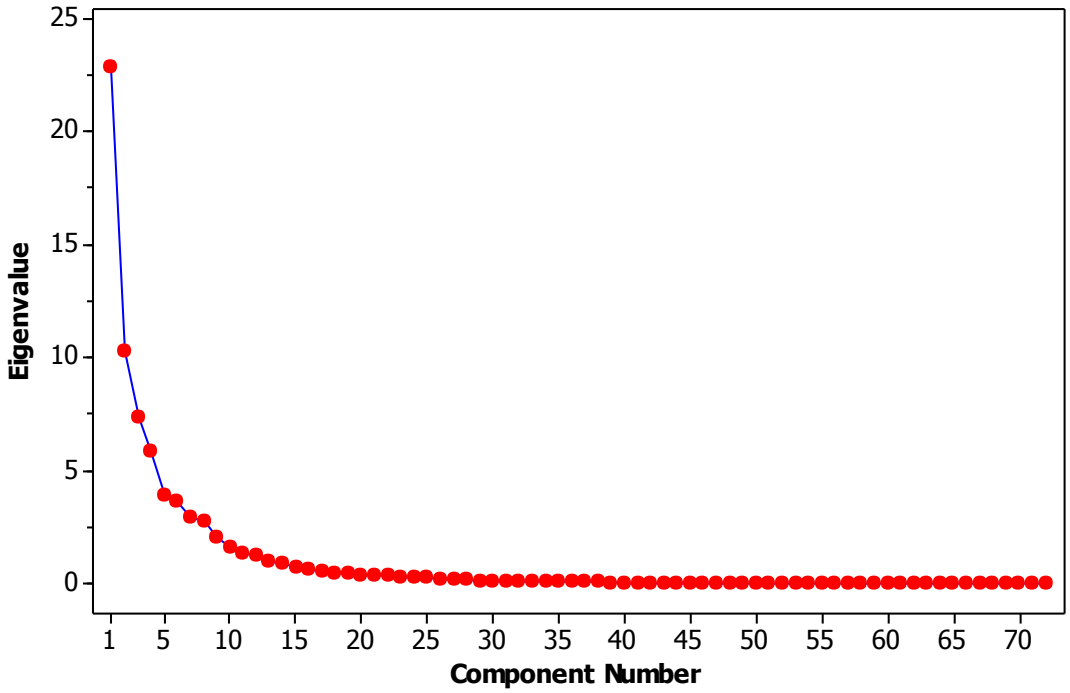

Figure 5. 16 Scree plot based on 72 variables expressing cattle behaviour after treatment

The results of the PCA based on 72 variables expressing cattle behaviour post-treatment are represented by a scree plot in Figure 5. 16. The plot shows that the first four principal components have eigenvalues above five and are larger than the other principle components which have smaller and closer matching eigenvalues.

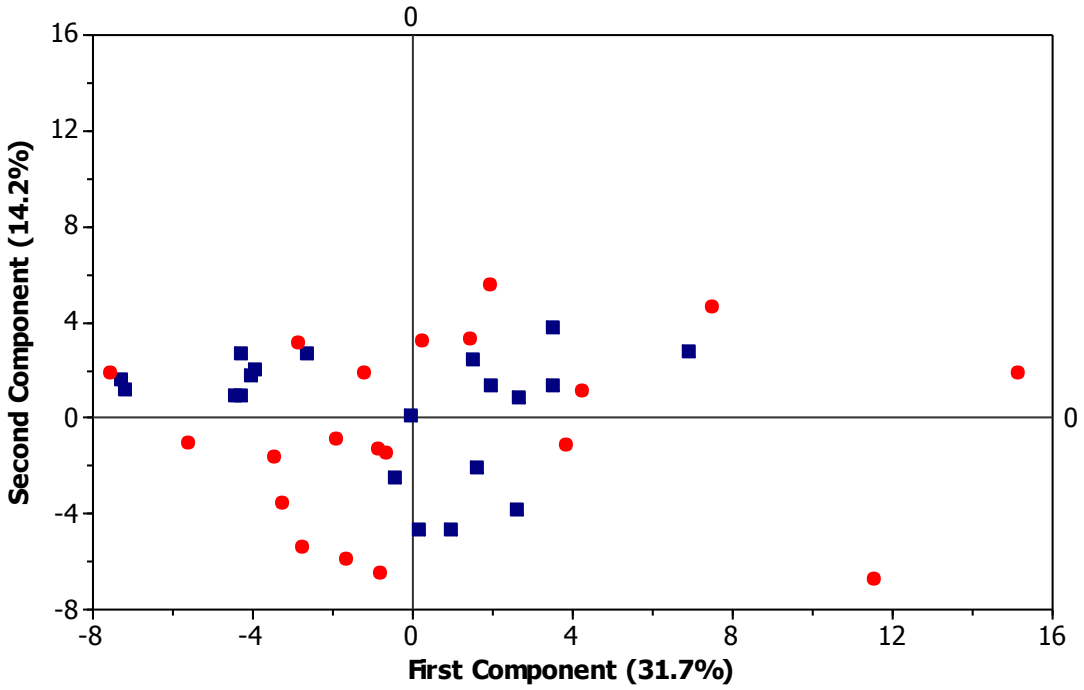

Figure 5. 17 Score Plot of the First and Second Principal components resulting from the PCA of post-treatment motion sensor data of cattle in the treated group (■) and control group (●) over a period of two weeks in Makale.

The post-treatment score plots were obtained from the PCA of data from the forty cattle that had motion sensors attached for two weeks after the treatment was carried out. Graphical representation of the first four PCs (accounting for 64.2% of overall data variability) resulted in six score plots. Figure 5. 17 and Figure 5. 18 represent score plots of second vs. first and second vs. third principal components resulting from the PCA of post-treatment motion sensor data respectively. Principal components one and two together accounted for 45.9% of the overall variability in the dataset (Table 5. 5); their score plot in Figure 5. 17 distinctly shows that the treated group of cattle has formed two clusters. The two clusters for treated cattle are more clearly defined in Figure 5. 18 which is a score plot of the second and third components that together account for 24.4% of the

variance. Control cattle in the two figures are evenly distributed and do not seem to form any discernible patterns.

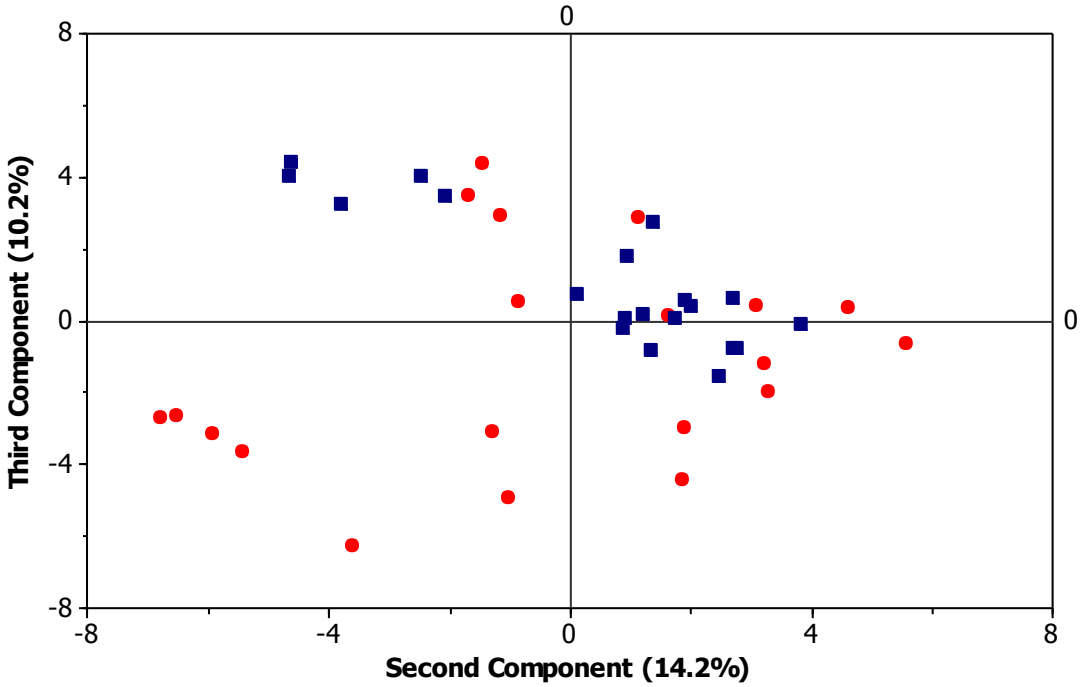

Figure 5. 18 Score Plot of the Second and Third Principal Components resulting from the PCA of post-treatment motion sensor data of treated group (■) and control group (●) cattle over a period of two weeks in Makale.

The score plots for post-treatment motion sensor data indicated that the treated group formed two distinct clusters while the control group was more evenly distributed. The score plots were generated from the first four principal components generated from the post-treatment data. Plotting the first four components against each other offered the best picture to examine for patterns. From the first four principal components, a total of six score plots were plotted (Appendix 4). Further analysis of the data was carried out by retaining the principal components that had eigenvalues greater than one. Table 5. 5 indicates that there were twelve PCs that had eigenvalues above one. This is also expressed graphically in the scree plot in Figure 5. 16 at the "elbow" of the graph.

The 12 PCs that had eigenvalues greater than one accounted for over 90% of the total variability in the data (Table 5. 5). The first twelve principal components and their loadings for the standing, walking and lying variables over 24 hours are displayed in Table 5. 6. The table only shows each movement behaviour variable's highest (absolute) loadings.

Table 5. 6 Principal components loadings for standing, walking and lying behaviour in Makale Veterinary Camp. Only each movement behaviour variable's highest (absolute) loadings are shown.

	Principal Component		*Principal Component*		*Principal Component*	
Time	*Standing*	*Loading*	*Walking*	*Loading*	*Lying*	*Loading*
0:00	1	0.189	2	-0.207	1	-0.189
1:00	1	0.189	3	-0.245	1	-0.188
2:00	1	0.18	3	-0.225	1	-0.181
3:00	1	0.177	12	0.347	1	-0.176
4:00	12	-0.194	12	-0.234	12	0.204
5:00	1	0.175	2	-0.238	1	-0.171
6:00	8	0.238	11	0.246	4	0.194
7:00	5	0.223	7	0.258	7	-0.352
8:00	5	0.23	2	-0.146	7	-0.282
9:00	2	0.211	6	-0.284	8	0.204
10:00	8	-0.199	10	-0.337	8	0.216
11:00	4	-0.291	10	-0.357	4	0.258
12:00	12	-0.233	10	-0.302	4	0.323
13:00	4	-0.248	4	-0.195	4	0.343
14:00	7	-0.18	3	0.217	4	0.277
15:00	7	-0.278	6	-0.247	7	0.38
16:00	11	0.191	6	-0.24	5	-0.276
17:00	11	-0.239	8	-0.273	5	-0.319
18:00	11	-0.252	10	-0.238	10	0.191
19:00	1	0.182	9	0.265	1	-0.183
20:00	1	0.177	9	0.225	1	-0.178
21:00	1	0.183	9	0.369	1	-0.183
22:00	1	0.189	12	-0.222	1	-0.19
23:00	1	0.19	3	-0.22	1	-0.19

The variables loading on principal component one fell into two groups within this principal component. The first group had positive principal component

loadings and highlighted standing behaviour in the night and early morning between the hours of 19:00 hours and 03:00 hours. The second group had negative principal component loadings and highlighted lying behaviour in the night and early morning between the hours of 19:00 hours and 03:00 hours. Because of the bipolarity in algebraic signs, it appears that the first component, which was the highest contributing factor at 31.7 %, was contrasting standing behaviour with lying behaviour in the night and early morning hours (Table 5. 7).

Table 5. 7 Movement behaviour variables influencing the first four principal components at different times of the day

	PC1 (31.7%)	*PC2 (14.2%)*	*PC3 (10.2%)*	*PC4 (8.1%)*
Standing	19:00 – 03:00hrs	09:00hrs	-	11:00,13:00hrs
Walking	-	Various morning hours	Various day & night hours	13:00hrs
Lying	19:00 – 03:00hrs	-	-	11:00 – 14:00hrs

Principal component 2 is dominated by a single positive loading for standing behaviour at 09:00 hours and three negative loadings for walking behaviour in the morning at 00:00 hours, 05:00 hours and 08:00 hours. This component had an overall contribution to data variability of 14.2% and appears to reflect the walking behaviour in the early morning hours contrasted with morning standing behaviour. Principal component 3, which contributed 10.2% of overall variability, was purely a component that was based on the walking behaviour at different times of the day. It contrasted walking behaviour in the night at 23:00 hours, 01:00 hours and 02:00 hours with walking behaviour during the afternoon hours at 14:00 hours. Principal component four had overall variability contribution of 8.1% and appeared to contrast the combination of standing and walking behaviour in the afternoon hours with lying behaviour during the same period.

Each of the remaining PCs had overall variability contribution of less than 6% and they each had different combinations of the three movement variables over the 24 hour period. The data in Table 5. 6 were graphically represented to show what behaviour variables were influencing which principal components and their corresponding loadings (Figure 5. 19).

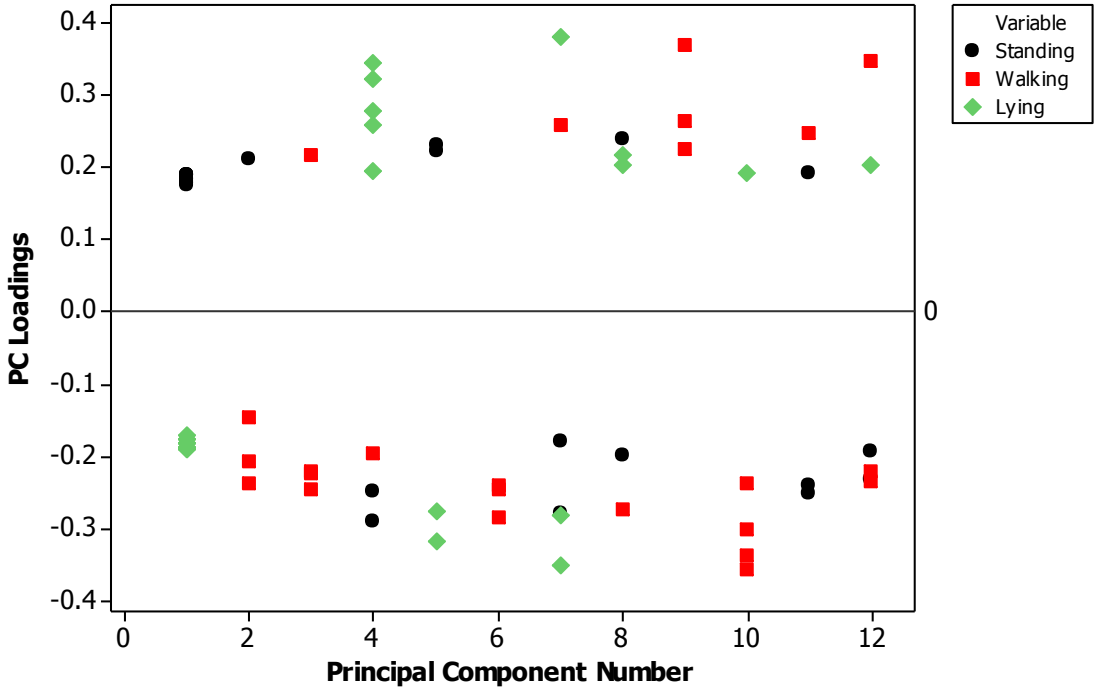

Figure 5. 19 Relationship of principal components (PC) and their corresponding loadings from the analysis of standing, walking and lying behaviour in Petauke District

The figure shows the varying importance of the three variables that contributed to the first twelve principal components (PCs). Each of the PCs shows which variables contributed the most to that individual PC. Principal component one was contrasting the difference in the lying and standing behaviour. The standing variables had positive loadings while lying variables had negative loadings. This principal component highlighted the fact that the greatest variability among the cattle was based on their standing and lying behaviour at different times of the day and night. Principal component two contrasted the walking (negative

loading) and standing (positive loadings) behaviour. Principal component three contrasted the walking (positive and negative loadings) behaviour of cattle during different times of the day. Principal component four contrasted the overall afternoon behaviour of cattle in Makale in terms standing (negative loadings), walking (negative loadings) and lying down (positive loadings). The remaining principal components reflected different combinations of the three movement behaviour variables and their loadings. The first four principal components described above accounted for 64.2% of overall data variability.

5.4 Discussion

The results in this study showed that a three co-administered broad-spectrum drug treatment improved low circulating haemoglobin levels in cattle to normal levels. Cattle that were in the treated group had significantly improved haemoglobin levels compared to control cattle ($p<0.001$). Improvement of circulating haemoglobin levels and condition have been demonstrated following treatment of animals by diminazene aceturate (Biryomumaisho, 2007; Van den Bossche et al., 2000). The treated group's mean haemoglobin profile improved from 6.94 g/dl at the pre-treatment stage to 8.14 g/dl at the post-treatment stage. The control group's mean haemoglobin levels remained below 8 g/dl both at the pre-treatment stage (6.87 g/dl) and post-treatment stage (7.14 g/dl). This meant that cattle in the control group remained below the 8 g/dl threshold and could be classified as anaemic (Schalm et al., 1975).

The movement behavioural patterns of cattle in the study were observed one week before and two weeks after treatment. The drugs used to treat against disease pathogens were diminazene aceturate (Berenil® - Intervet), long-acting oxytetracycline (Oxyject LA® — Dopharma, Netherlands) and albendazole (Dopharma, Netherlands). This reflected the normal management practice in the area and the drugs are used to treat trypanosomiasis, tick borne and pasture-transmitted diseases. The numbers of steps taken by cattle were one of the parameters used to study these behavioural patterns. There were no significant differences between the mean numbers of steps of control and treated groups of cattle at the pre-treatment stage. This was as expected because animals were from the same area and under a similar cattle management system (Chapter 3). Additionally, the animals faced the same disease challenges in the area and this

was reflected by similar haemoglobin values at the pre-selection stages. At the post-treatment stage of the experiment, the treated group had significantly higher step counts than the control group. The difference in haemoglobin levels mentioned earlier might have contributed to enabling the treated group to maintain higher step counts while the control group of animals suffered the effects of lower circulating haemoglobin levels.

Treated cattle showed a significant difference between the number of steps taken in the week before treatment and the increased step counts of the second week after treatment. This group saw their mean haemoglobin levels improve probably due to the drug administration. The improved haemoglobin levels may have aided the animals in the treated group have mean step counts rise from 6,697 steps per day one week before treatment to 8,131 steps per day two weeks after treatment. Cattle in the control group did not show any significant difference during the same period. There was no significant difference between the number of steps taken in the week before treatment and the first week after treatment for both treated and control cattle. Significant numbers of steps were noticed for cattle in the treated group during the second week. Treated cattle showed a 17% increase in step counts during the second week compared to a 2.6% increase by control cattle. During the first week after treatment, animals did not show any marked increase in the number of steps as the drugs might have still needed time to work. Physiologically, the animals needed time in which to respond to the treatment before getting better during the period of convalescence.

During the second week after treatment, not all animals responded by having increased step counts. It is possible that some animals may not have responded

to treatment after two weeks because they needed more time to respond or possibly because of drug resistance especially against diminazene aceturate. It has been shown that there has been five-fold increase in *T. congolense* isolates resistant to diminazene aceturate in Eastern Zambia from 1996 to 2003 (Delespaux et al., 2008). Since there were no indications that the drug pressure increased between 1996 and 2003, it was suggested that genetic exchange of resistance genes might explain the increased frequency of resistance to diminazene aceturate (Delespaux et al., 2008). It would have been interesting to see how long animals with increased step counts would have sustained them if the experiment had carried on longer than the two week period. It would also have been interesting to see if all treated animals would have increased their steps counts if they had been monitored for a longer period.

The results showed that there were no differences in the mean number of steps taken between the treated and control groups at the start of the cattle study ($p=0.71$) in the week before treatment. At this stage of the experiment, there were 8 cattle in the treated group that had significantly more number of steps taken per day than control cattle (Figure 5. 2). There were 7 cattle in the control group that had significantly more number of steps taken per day than treated cattle. That there were no significant differences between the two groups was as expected because animals were from the same area and under a similar cattle management system.

In the week following treatment, control cattle took significantly more steps than treated cattle ($p<0.05$). There were only 2 cattle in the treated group that had significantly more steps taken per day than control animals during the week after treatment. There were 10 cattle in the control group that had significantly

more number of steps taken per day in the first week after treatment (Figure 5. 3). This could have been because the treated animals were starting to respond to the treatment before any improvement could be noticed. During the first week after treatment, treated animals did not show any marked increase in the number of steps as the drugs might have still needed time to work. Physiologically, the animals might have needed time in which to respond to the treatment before getting better during the period of convalescence.

In the second week after treatment, the treated group of cattle took significantly more steps than the control group ($p<0.5$). At this stage of the experiment, there were 7 cattle in the treated group that had significantly more number of steps taken per day. There were 2 cattle in the control group that had significantly more number of steps taken per day in the second week after treatment (Figure 5. 4). At this stage of the experiment, cattle in the treated group saw a 17% increase in the number of steps from the pre-treatment stage while the control group of animals had a 2.6% increase (Table 5. 3).

These investigations have confirmed that step counts of motion sensors may be used to investigate movement behaviour in traditionally managed cattle, and that whereas reduced haemoglobin levels associated with various parasitic infections may suppress cattle movement activity, treating these infections improves haemoglobin levels and may improve or restore more normal movement behaviour patterns. Cattle step counts of non-treated cattle in this study were similar to cattle step count results of Makale low haemoglobin cattle obtained during the 2006/07 study (Chapter 4). This study demonstrated the repeatability of the experiment. This may have been because the study was conducted in the same area during the same season in the year. The body

condition scores did not significantly differ between treated and control cattle. This may have been because of the abundant availability of grass for grazing that is common during the rainy season.

The daily group patterns of treated and control cattle in the study were found to be similar showing increased walking and standing during the day when the animals were grazing and being herded. There was increased lying down during the night when the animals were resting. Clear patterns of cattle movement behaviour over a twenty four hour period were observed. Standing behaviour for both the treated and control groups of animals started around the same time at 06:00hrs in the morning until around 17:00 hours in the evening when it started to decline. This behaviour was as expected because this was the normal period that cattle were grazing and being herded during the day.

The walking behaviour profiles for the treated and control groups were not very different. Animals in both groups started the day at around 04:00hrs and stopped almost all walking behaviour by 19:00hrs. Overall there was a slight decrease in walking behaviour between 12:00hrs and 13:00hrs. This corresponded with a slight increase in lying behaviour around the same time. This appeared to be an indication that animals were resting at this time of day. Animals around this time of the day were seen resting under the shades of trees as it was very hot (Personal observation). The decrease in walking behaviour around midday created two walking peaks in the morning and late afternoon (Figure 5. 9). These walking peaks might also have arisen due to the cattle being herded out to graze in the mornings and brought back to the kraals in the evening (Chapter 3).

Standing behaviour for control cattle appeared to be more variable than behaviour for cattle in the treated group. This variability was greatest during the night time hours between 19:00 hours and 06:00 hours (Figure 5. 8). The variability during the day time hours between the two groups appeared to be the same. The variability exhibited in the walking behaviour profile appeared to be similar for both the treated and control groups. Lying behaviour profiles showed that the control cattle group had greater variability than the treated group during night time hours between 19:00 hours and 06:00 hours (Figure 5. 9).

Mean cattle time budgets in Petauke District revealed the percentage of time cattle spent standing, walking or lying down during the whole duration of the study (Figure 5. 11 and Figure 5. 12). At the pre-treatment stage, the time budgets revealed that in a twenty four hour period, cattle in the treatment group spent 49% of their time standing and 40% lying down. The remainder of the time (11%) was spent walking. Cattle in the control group spent 50% standing, 38% lying down and 12% walking.

At the post-treatment stage during week one, control cattle spent more time walking than cattle in the treated group. These results support the cattle step count results earlier discussed in which it was found that control cattle took significantly more steps than treated cattle one week after treatment. In the second week post-treatment, treated cattle spent more time walking ($p < 0.05$) and lying down ($p < 0.001$) than control cattle. On the other hand, control cattle spent more time standing during the week than those in the treated group ($p < 0.001$).

A principal components analysis was also used to further analyse the data. The PCA revealed that there were differences in the behaviour of treated and control cattle based on their movement patterns. The score plots from the PCA based on 72 variables expressing cattle behaviour in Makale Veterinary Camp revealed two distinct clusters within the treated group while the control group cattle were evenly distributed and showed no distinct clusters. This pattern was a reflection of the underlying differences in cattle behaviour between the treated cattle and the control group. The PCA showed that the greatest difference between the behaviours of the two groups was in their standing and lying behaviour (Figure 5. 19). Animals that were treated and ended up having higher haemoglobin levels were found to be closer together on the score plots. This may be because they exhibited "normal" behaviour while the control cattle that had lower haemoglobin levels (and therefore might have been sick animals) were more scattered on the score plots implying the more erratic behaviour of weak or stressed animals. The level of anaemia (low haemoglobin) or the PCV usually gives a reliable indication of the disease status and productive performance of an infected animal (Trail et al., 1991). Cattle with chronic forms of trypanosomiasis have been reported to be lethargic and anaemic with low levels of circulating haemoglobin (Naessens, 2006; Sekoni et al., 1990).

Two-dimensional score plots and loading profiles of the principal components (PC) were used to visualise the relative contribution of individual movement behaviour variables to the clustering of the different animals. Score plots from the principal components of cattle in the treated and control groups during the post-treatment stage revealed separate clusters of cattle from the two groups. The principal components loading profiles indicated that clustering was mainly based on the differences in night time standing and lying behaviour between the

treated and control groups. Treated animals spent more time lying down and control cattle spent more time standing. Cattle lying behaviour has been used as a measure of cattle well-being in the dairy industry (Cook et al., 2005; Robert et al., 2009) suggesting that animals that are at ill at ease will spend more time standing. The PCA through the various methods employed in this chapter appears to have teased out the qualitative and quantitative distinctions in the data and suggested that administering a broad spectrum of drugs reduced variability among cattle in terms of movement and that the reduction in variability related to movement behaviour at particular times of the day.

CHAPTER 6

Clinical characteristics and molecular parasitology of traditionally managed cattle in movement behaviour studies

in Petauke District

6.1 Introduction

The studies described in this chapter investigate the clinical characteristics of traditionally managed cattle before and after a treatment. The parasitological status of cattle prior to the intervention in the two areas is also determined. Clinical data collection included measurement of haemoglobin and rectal temperature as objectively verifiable indicators for disease. Other clinical parameters measured were weight, lymph node size, condition of mucous membranes, presence of discharges, presence of diarrhoea, condition of hair coat, presence of skin lesions and body condition score (Chapter 2, Section 2.1.3). Molecular parasitological tests were carried out using the polymerase chain reaction (PCR) for the presence of *Trypanosoma* and *Theileria* parasites. Motion sensor step counts were used to determine cattle movement.

6.2 Materials and methods

6.2.1 Study area

The clinical and molecular parasitological data presented in this chapter were collected during the baseline study in Makale and Kasero Veterinary Camps in 2006/07 (Chapter 4) and also during the treatment study conducted in Makale Veterinary Camp in 2008 (Chapter 5).

6.2.2 Sensitisation of farmers

Farmers were informed of clinical data and sample collection procedures 48 hours before commencement through the Petauke District veterinary office as outlined in Chapter 4 (Section 4.2.1.2) and Chapter 5 (Section 5.3.1.1).

6.2.3 Study design

6.2.3.1 Cattle selection

Cattle owners or their herdsmen presented a total of 636 cattle during the baseline and treatment studies. The number of cattle clinically examined and screened for blood during the baseline study in Makale and Kasero in 2006/07 was 432 while that for the treatment study in Makale in 2008 was 204. In both studies, a total of 40 cattle were selected for motion sensor attachment with selection of the animals being specific to each of the studies as outlined in chapter 4 (Section 4.2.2) and chapter 5 (Section 5.3.2).

Previous studies conducted in Petauke district in 2004 showed the mean molecular parasitological prevalence of trypanosomiasis in Makale was 22.5% and that for *Theileria parva* in Kasero was 32.5% (Mubanga, 2009). Sampling rates were selected that were able to detect at least one infected animal at a given level of confidence given a certain prevalence of infection. Published sample size tables were used to calculate the sample sizes required to attain 95% confidence intervals around prevalences of 22.5% for Makale and 32.5% for Kasero (Thrusfield, 1997). A sample size of 200 animals was chosen for Kasero which produced a 95% confidence interval of 24% to 36% prevalence values which included the estimated prevalence of 32.5% for *Theileria parva* in this area. For Makale, a sample size of 200 was chosen which produced a 95% confidence interval of 14% to 26% prevalence values which included the estimated prevalence of 22.5% for trypanosomiasis in this area. To improve on the reliability of the sample sizes, more than the required numbers of cattle were screened in both two veterinary camps. As a result in Kasero, a total of 211

animals were sampled while 221 were sampled in Makale during the 2006/07 study. In the 2008 Makale treatment study, 204 animals were sampled.

6.2.3.2 Measurement of clinical parameters, blood specimen collection and recording motion sensor data

Measurement of clinical parameters and blood specimen collection was carried out for the 432 animals in the baseline study of 2006/07 as explained in Chapters 2 and 4. The blood specimens collected in the baseline study were immediately used pen-side for haemoglobin measurement and some stored on Whatman FTA® cards for molecular parasitological testing later in the laboratory. The 204 cattle selected during the treatment study in 2008 were clinically examined as earlier explained in chapter 5. Blood was also collected from these animals for haemoglobin measurement using a haemoglobinometer. Pedometer data was recorded and collected as outlined in Chapter 4 (Section 4.2.3).

6.2.3.3 Detection of *theileria* and trypanosome parasites using molecular parasitological techniques

The laboratory detection of trypanosome and theileria parasites employed the use of molecular parasitological methods. The polymerase chain reaction (PCR) was used to detect the p104 gene for *Theileria parva* using forward and reverse primers that generated a product of 278bp (Iams et al., 1990; Skilton et al., 2002). Species specific primers for *T. congolense* (Savannah type), *T. vivax* and *T. Brucei* were used for amplification of the pathogens in the field samples (Masake et al., 1997; Masiga et al., 1992; Moser et al., 1989).

6.3 Data recording and storage

Clinical data resulting from the clinical examination of the 636 animals during the baseline and treatment studies was recorded onto the field record sheets (Chapter 2, Table 2.1). Clinical data that was obtained from the 80 animals during the pedometer attachment period in both studies was recorded onto cattle data collection forms (Chapter 4, Table 4.2). All data recorded on paper forms was later transferred to a Microsoft Excel (2003) workbook for storage and future analyses.

6.4 Results

The results shown in this section are those from the baseline study (432 cattle) during 2006/07 and from the treatment study (204 cattle) during 2008. The baseline study conducted in 2006/07 had a pre-intervention component only while the treatment study carried out in 2008 had both a pre-intervention component and a post-intervention one (Table 6. 1). The results include clinical and parasitological data obtained during the baseline and treatment studies. Clinical and parasitological data is also included for the motion sensor attachment period in both studies. Motion sensor data in the form of step counts is presented for both the baseline and treatment studies.

Table 6. 1: Pre and post-intervention components of the baseline and treatment studies

	Baseline study 2006/07	*Treatment study 2008*
Pre-intervention	Yes	Yes
Post-intervention	No	Yes

6.4.1 Molecular parasitology of cattle in the Baseline Study (2006/07)

The molecular diagnosis of *Theileria parva (T. parva)*, *Trypanosoma congolense (T. congolense)* (Savannah type), *Trypanosoma vivax (T. vivax)* and *Trypanosoma brucei (T. brucei)* was carried out at the University of Edinburgh by polymerase chain reaction (PCR) using species specific primers (Chapter 2, section 2.2.9). Molecular Parasitology results were obtained by carrying out the polymerase chain reaction on 552 samples on FTA cards from Makale and Kasero during the 2006/07 baseline study. A total of 432 samples were from the pre-selection stage while 120 samples were from 40 animals that had pedometers attached. Animals were sampled on the day of motion sensor attachment (day 0), on days 7 and 14.

6.4.1.1 Kasero Veterinary camp - 2006/07

The number of animals that tested positive for *T. parva*, *T. congolense*, *T. vivax* and *T. brucei* is shown in Table 6. 2. There were no cases of *T. vivax* or *T. brucei* detected in Kasero by PCR. A total of 54 cattle were positive by PCR for *T. parva* representing 25.6 %, 95 % [CI 20.2, 31.9] of sampled cattle. The *T. parva* amplicon size generated at the end of the PCR was 276 bp (Figure 6. 1). The number of animals that were positive for *T. congolense* by PCR in Kasero was only 3 representing 1.4 %, 95% [CI 0.5, 4.1 %] of sampled cattle.

Table 6. 2 Percentage of cattle positive for trypanosomiasis or *T. parva* in Kasero (2006/07 baseline study)

	Kasero			
	T. parva	***T. congolense***	***T. brucei***	***T. vivax***
n	211	211	211	211
Positive	54	3	0	0
Percentage	25.6 %	1.4 %	0 %	0 %
95 % CI	20.2 - 31.9 %	0.5 – 4.1 %	-	-

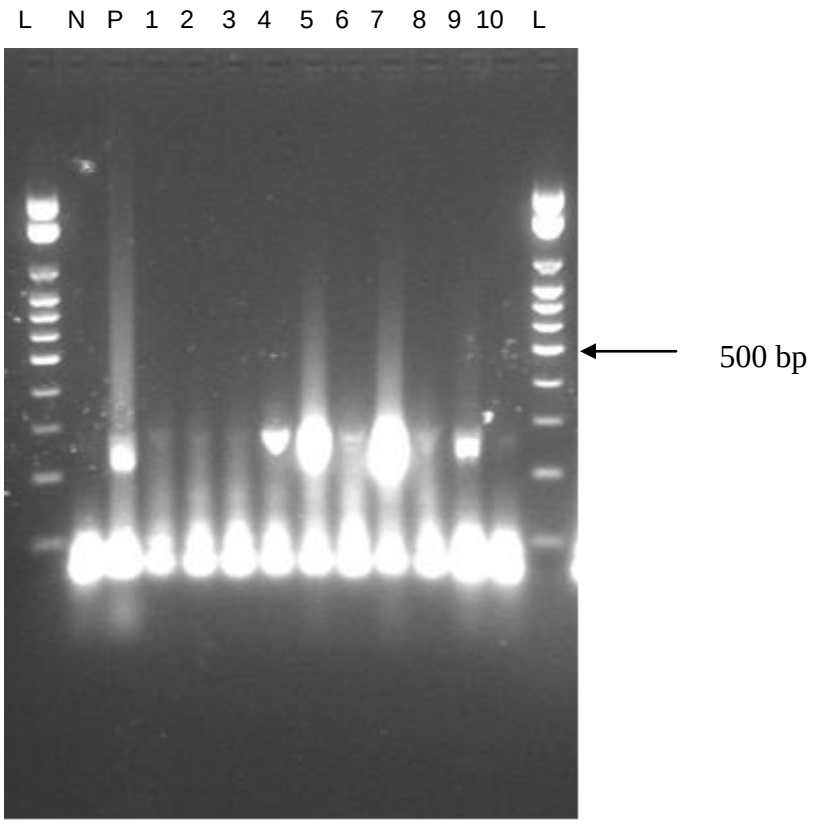

Figure 6. 1 PCR bands of *T. parva* from Petauke District, Zambia. L = maker (100bp ladder), P (positive control = *T. parva*), N (negative control) and Lines 1- 10 are field samples (amplicon size 276bp).

The twenty animals that had pedometers attached to their hind legs had blood taken for molecular parasitological analysis at the pre-selection stage, on day 0, day 7 and day 14 of the study. Table 6. 3 shows the PCR results for pedometer cattle pairs that were tested for *T. parva* in Kasero. A total of six animals that had pedometers attached were ever positive at any one time during the study. All six of these animals tested positive for *T. parva* at the pre-selection stage. Three of these animals belonged to the high haemoglobin group of animals while the other three were in the low haemoglobin group. There were no animals that had pedometers attached that tested positive for *T. congolense* in Kasero. From the twenty animals, a total of 80 blood samples were tested for *T. parva* during the study. Twenty of the samples (25 %) tested positive for *T. parva.* Three of the

animals tested were positive on all four occasions of being tested at pre-selection, day 0, day 7 and on day 14. Five of the animals were positive on at least three occasions of being tested while six of the animals were positive at least twice during the study. If an animal ever tested positive, it was again found positive on at least one more occasion later in the study.

Table 6. 3 *T. parva* PCR results for cattle pairs that had pedometers in Kasero (2006/07 baseline study)

Ear Tag Number	Hb Status	Stage of study				Total Number +ve	Mean Hb Value (g/dl)
		Pre-Selection	Day 0	Day 7	Day 14		
35	High	P	P	P	P	4	13.8
109	Low	N	N	N	N	0	9.2
8	High	P	N	P	P	3	13.5
19	Low	N	N	N	N	0	9.2
81	High	N	N	N	N	0	13.3
15	Low	N	N	N	N	0	10.1
11	High	N	N	N	N	0	15
61	Low	N	N	N	N	0	9.8
72	High	N	N	N	N	0	13.5
42	Low	N	N	N	N	0	9.6
103	High	P	N	N	P	2	12.9
193	Low	P	P	P	P	4	8
149	High	N	N	N	N	0	13.5
195	Low	P	P	P	P	4	8.4
16Y	High	N	N	N	N	0	14.4
178	Low	P	P	N	P	3	8.3
167	High	N	N	N	N	0	14.9
104	Low	N	N	N	N	0	9.0
130	High	N	N	N	N	0	15.2
154	Low	N	N	N	N	0	8.9
Total Positive		**6**	**4**	**4**	**6**	**20**	

P = Positive, N = Negative

Mean haemoglobin for all 20 cattle = 11.5 g/dl

Mean haemoglobin for high haemoglobin group of cattle = 14 g/dl

Mean haemoglobin for low haemoglobin group of cattle = 9 g/dl

6.4.1.2 Makale veterinary camp - 2006/07

The number of animals that tested positive for *T. parva*, *T. congolense* (Savannah), *T. vivax* and *T. brucei* is shown in Table 6. 4. There were no cases of *T. vivax* or *T. brucei* detected by PCR in Makale. A total of only 5 cattle were positive by PCR for *T. parva* representing 2.4 %, 95 % [CI 1.0, 5.2] of sampled cattle. The number of animals that were positive for *T. congolense* by PCR in Makale was 47 representing 21.4 % [16.4, 27.1] of sampled cattle. The *T. congolense* amplicon size generated at the end of the PCR was 316 bp (Figure 6. 2).

Table 6. 4 Percentage of cattle positive for theileriosis or trypanosomiasis in Makale (2006/07 baseline study)

	Makale			
	T. parva	***T. congolense***	***T. brucei***	***T. vivax***
n	221	221	221	221
Positive	5	47	0	0
Percentage	2.4 %	21.4 %	0 %	0 %
95 % CI	1.0 – 5.2 %	16.4 – 27.1 %	-	-

Table 6. 5 shows the PCR results for pedometer cattle pairs that were tested for *T. congolense* in Makale. A total of eight animals that had pedometers attached tested positive for *T. congolense* at the pre-selection stage. All of these animals belonged to the low haemoglobin group of animals while there were none from the high haemoglobin group. There were no animals that had pedometers attached that tested positive for *T. parva* in Makale. From the twenty animals, a total of 80 blood samples were tested for *T. congolense* during the study. Twenty nine of the samples (36.25 %) tested positive for *T. congolense (*Table 6. 5*).*

Table 6. 5 *T. congolense* PCR results for cattle pairs that had pedometers in Makale (2006/07 baseline study)

Ear Tag Number	*Hb Status*	*Stage of study*				Total Number +ve	*Mean Hb Value (g/dl)*
		Pre-Selection	Day 0	Day 7	Day 14		
33	High	N	N	N	N	0	13.3
46	Low	P	P	N	P	3	5.0
41	High	N	N	N	N	0	13.3
50	Low	P	P	P	P	4	5.8
196	High	N	N	N	N	0	12.7
81	Low	P	P	P	P	4	5.9
58	High	N	N	N	N	0	10.8
90	Low	P	P	P	P	4	7.4
61Y	High	N	N	N	N	0	12.2
154	Low	N	N	N	N	0	6.6
133	High	N	N	N	N	0	10.1
125	Low	P	N	P	P	3	6.4
20Y	High	N	N	N	N	0	12.5
3	Low	P	P	P	P	4	6.5
129	High	N	N	N	N	0	13.1
70Y	Low	P	P	P	P	4	6.4
86	High	N	N	N	N	0	12.9
137	Low	N	N	N	N	0	10.7
91	High	N	N	N	N	0	10.7
156	Low	P	P	NA	P	3	6.6
Total Positive		**8**	**7**	**6**	**8**	**29**	

P = Positive, N = Negative, NA = Not available
Mean haemoglobin for all 20 cattle = 9.4 g/dl
Mean haemoglobin for high haemoglobin group of cattle = 12.2 g/dl
Mean haemoglobin for low haemoglobin group of cattle = 6.7 g/dl

Five of the animals tested were positive on all four occasions of being tested at the pre-selection stage, on day 0, day 7 and on day 14. Eight of the animals were

positive on at least three occasions of being tested (Table 6. 5). If an animal ever tested positive, it was again found positive on at least two more occasions later in the study.

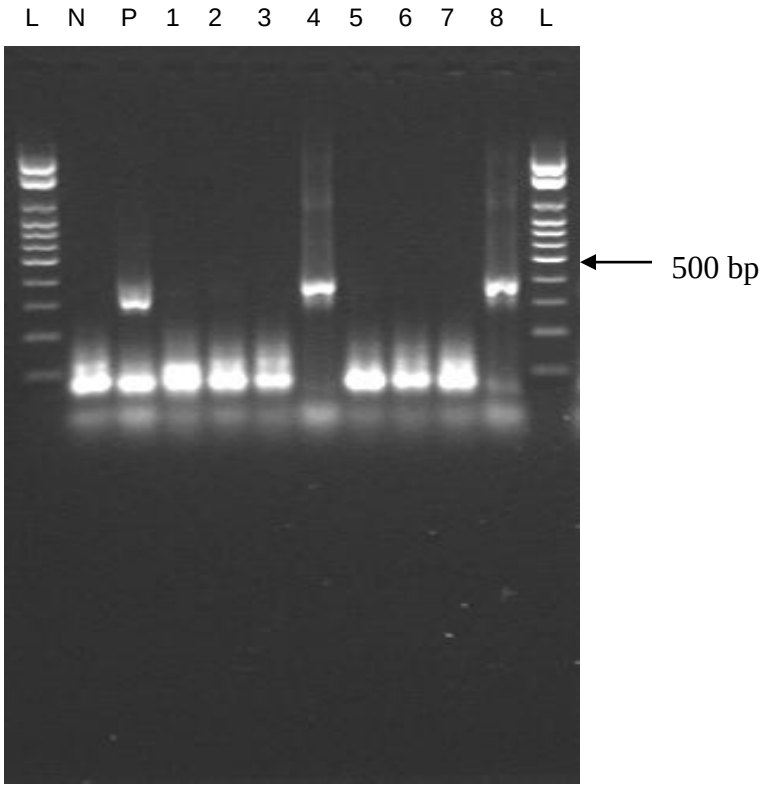

Figure 6. 2 PCR bands of *T. congolense* from Petauke District, Zambia L=maker (100bp ladder), P (Positive control = *T. congolense*), N (Negative control) and Lines 1- 8 are field samples (Amplicon size 316 bp).

6.4.2 Movement behaviour of cattle that were positive by molecular parasitology

6.4.2.1 Step counts for *T. parva* positive cattle in Kasero

There were five pairs of co-grazing cattle in Kasero that had at least one animal positive for *T. parva* (Table 6. 3). Four pairs had one animal in the pair positive for *T. parva* and the other negative. One co-grazing pair had both animals in the pair positive for *T. parva* and was excluded from the current analysis. Five pairs

that had all animals test negative for *T. parva* were also excluded from the current analysis. Figure 6. 3 represents the mean daily steps of the four pairs of cattle that had one animal positive for *T. parva* and the other negative.

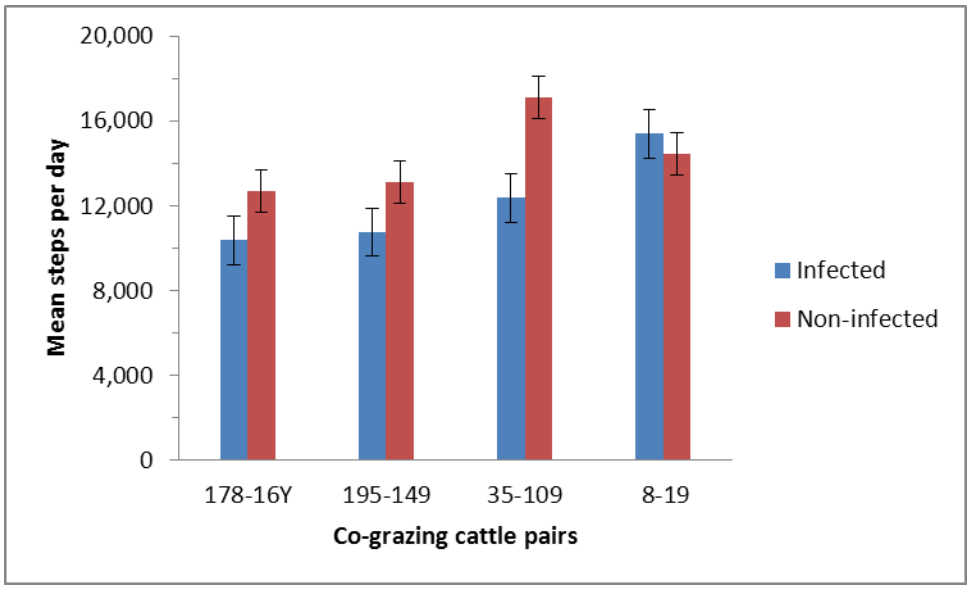

Figure 6. 3 Mean daily steps of *T. parva* infected and non-infected co-grazing cattle pairs in Kasero. Error bars represent standard error for the data.

Three of the cattle that were infected with *T. parva* had fewer steps than the non-infected cattle over the two week period. One of the infected animals had significantly lower daily step counts than their non-infected partners (Figure 6. 3). The mean daily numbers of steps taken by the infected group of cattle was 12,221 steps while that for the non-infected group was 14,114 steps. However, there was no overall significant difference between the mean numbers of steps of the *T. parva* infected and non-infected cattle in Kasero (paired t-test; difference of means = - 1893, 95% CI [-5857, 1618], p = 0.17).

6.4.2.2 Step counts for *T. congolense* positive cattle in Makale

There were eight pairs of co-grazing cattle in Makale that had at least one animal test positive for *T. congolense* (Figure 6. 4). Two pairs of cattle had all animals test negative for *T. congolense* and were excluded from the current analysis. Figure 6. 4 represents the mean daily steps of the eight pairs of cattle that had one animal positive for *T. congolense* and the other negative.

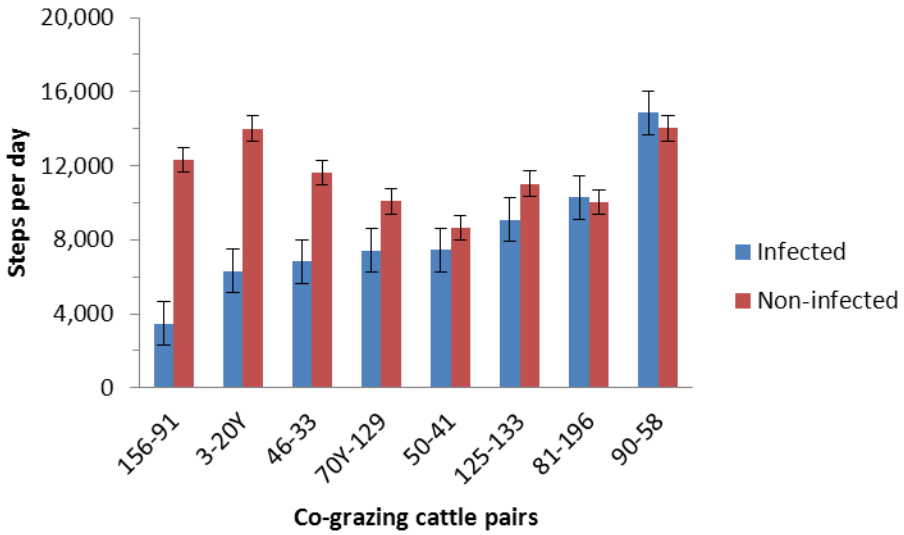

Figure 6. 4 Mean daily steps of *T. congolense* infected and non-infected co-grazing cattle pairs in Makale. Error bars represent standard error for the data.

Six of the cattle that were infected with *T. congolense* had fewer steps than the non-infected cattle over the two week period. Four of the infected animals had significantly lower daily step counts than their non-infected partners (Figure 6. 4). The mean daily numbers of steps taken by the infected group of cattle was 8,211 steps while that for the non-infected group was 11,469 steps. There was a significant difference between the mean numbers of steps by the *T. congolense* infected and non-infected cattle in Makale (paired t-test; difference of means = -3258, 95% CI [-6240, -276], $p < 0.05$).

6.4.3 Clinical data

6.4.3.1 Petauke baseline study 2006/07

Table 6. 6 and Table 6. 7 show descriptive statistics for the haemoglobin, rectal temperature, weight and age results of all animals sampled in the pre-selection stage of the baseline study conducted in 2006/07 in Kasero and Makale veterinary camps respectively. The mean haemoglobin value for the 211 animals sampled in Kasero was 10.4 g/dl while their mean temperature was 39.1 ºC. Animals in Kasero had average weight of 225 Kg and mean age of 4.4 years.

Table 6. 6 Clinical parameters for Kasero Cattle at pre-selection stage (2006/07 baseline study)

Variable	*N*	*Mean*	*Median*	*StDev*	*Min*	*Max*
Hb (g/dl)	211	10.4	10.2	1.6	6.9	16.7
RT (ºC)	211	39.1	39.2	0.6	34.9	40.7
Weight (Kg)	211	225	230	77	47	530
Age (Years)	211	4.4	4.0	2.3	0.25	15.0

Hb = Haemoglobin (g/dl), RT = Rectal Temperature (ºC)

Table 6. 7 Clinical parameters for Makale Cattle at pre-selection stage (2006/07 baseline study)

Variable	*N*	*Mean*	*Median*	*StDev*	*Min*	*Max*
Hb (g/dl)	221	9.4	9.4	2.2	4.2	14.7
RT (ºC)	221	38.9	38.9	0.5	37.4	40.5
Weight (Kg)	221	224	230	98	42	560
Age (Years)	221	4.2	4.0	2.6	0.30	12.0

Hb = Haemoglobin (g/dl), RT = Rectal Temperature (ºC)

In Makale, a total of 221 animals were screened in the baseline study. The mean haemoglobin value for Makale was 9.4 g/dl while their mean rectal temperature was 38.9 ºC. The average weight in Makale was 224 Kg while their average age

was 4.2 years. Makale recorded the animal with the lowest haemoglobin level at 4.2 g/dl while Kasero recorded the animal with highest haemoglobin level at 16.7 g/dl in the study (Figure 6. 5). The highest rectal temperatures recorded in Kasero and Makale were 40.7 ºC and 40.5 ºC respectively. The difference in mean haemoglobin values recorded for cattle sampled in Kasero (10.4 g/dl) and Makale (9.4 g/dl) at the pre-selection stage was found to be highly significant (2-sample t-test; estimate for difference = 1.02, 95 % CI [0.65, 1.40], $p < 0.001$).

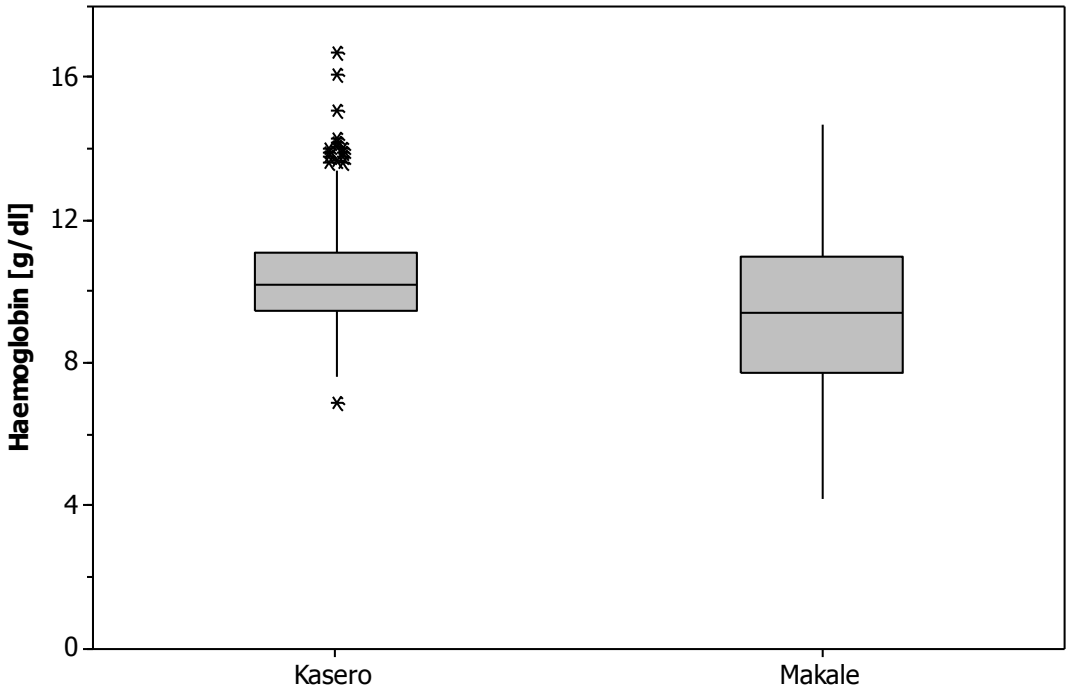

Figure 6. 5 Haemoglobin levels of Kasero and Makale cattle in the baseline study. Each box indicates the lower and upper quartiles representing the haemoglobin values below which 25 % and 75 % of the observations lie respectively. The median haemoglobin value is shown within each box as a horizontal line. This is the value below which half, and above which half, the observations lie. The whiskers show the range of observations excluding outliers, which are extreme haemoglobin values represented by asterisks in the figure. [Kasero, n = 211, Makale, n = 221]

6.4.3.1.1 Clinical parameters of *T. parva* positive and negative cattle

A total of 59 cattle (13.7 %) tested positive for *t. parva* by polymerase chain reaction in the baseline study as a whole. However, Makale only had 5 cattle (2.3 %) testing positive for *T. parva* while Kasero had 54 cattle (25.6 %) testing positive for *T. parva*. The haemoglobin levels of *T. parva* positive animals were slightly lower than those for *T. parva* negative animals (Figure 6. 6).

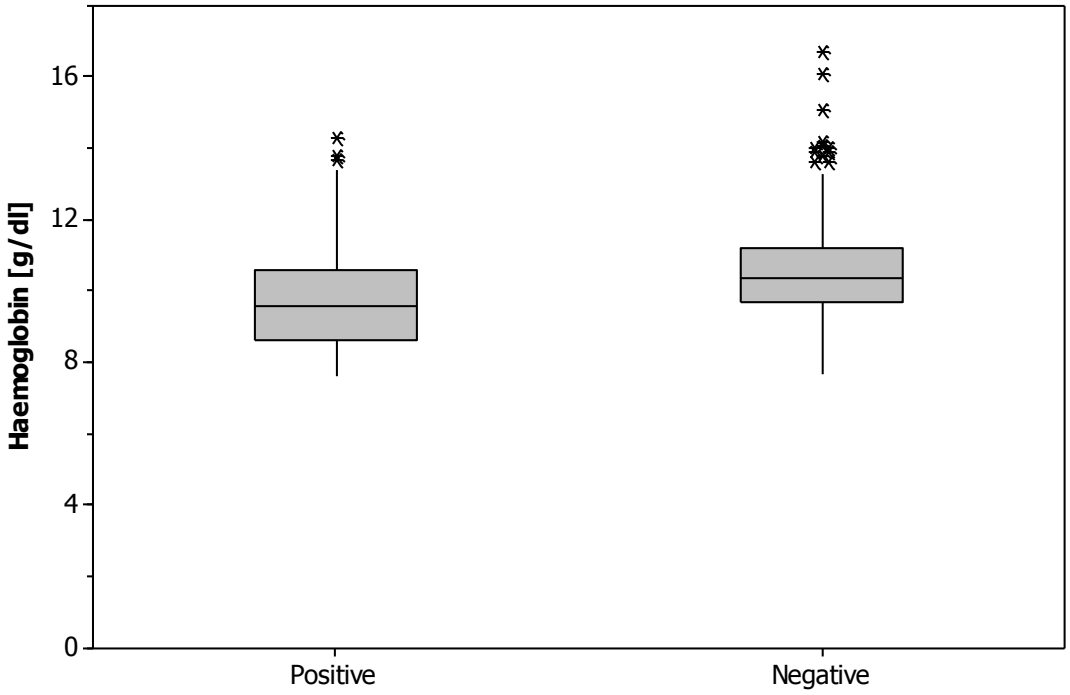

Figure 6. 6 Haemoglobin levels of *T. parva* PCR positive and negative cattle in Kasero

Animals that tested positive for *T. parva* by PCR in Kasero had a mean haemoglobin value of 9.8 g/dl (Table 6. 8). Those animals that tested negative for the same pathogen had a mean haemoglobin value of 10.7 g/dl (Table 6. 9). The difference in mean haemoglobin values between the *T. parva* positive and negative cattle was found to be highly statistically significant (2-sample t-test; difference of means = -0.90, 95 % CI [-1.40, -0.39], p< 0.001). The haemoglobin

range for *T. parva* positive animals was between 6.9 and 14.3 g/dl while that for the negative animals was from 7.7 to 16.7 g/dl (Figure 6. 6).

Table 6. 8 Clinical parameters of *T. parva* positive cattle in Kasero (2006/07 baseline study)

Variable	*N*	*Mean*	*Median*	*StDev*	*Min*	*Max*
Hb (g/dl)	54	9.8	9.6	1.6	6.9	14.3
RT (ºC)	54	39.0	39.1	0.9	34.9	40.1
Weight (Kg)	54	208	220	67	67	408
Age (Years)	54	4.5	4.0	2.7	0.5	15

Hb = Haemoglobin (g/dl), RT = Rectal Temperature (ºC)

Table 6. 9 Clinical parameters of *T. parva* and *T. congolense* negative cattle in Kasero (2006/07 baseline study)

Variable	*N*	*Mean*	*Median*	*StDev*	*Min*	*Max*
Hb (g/dl)	155	10.7	10.4	1.5	7.7	16.7
RT (ºC)	155	39.2	39.2	0.5	36.2	40.7
Weight (Kg)	155	231	240	79	47	530
Age (Years)	155	4.3	4.0	2.0	0.2	12.

Hb = Haemoglobin (g/dl), RT = Rectal Temperature (ºC)

The animal that had the lowest haemoglobin reading (6.9 g/dl) in Kasero belonged to the *T. parva* positive group while the *T. parva* negative group had the animal with the highest recorded haemoglobin value (16.7 g/dl) in Kasero. The mean rectal temperatures for Kasero negative and positive cattle were 39.0 ºC and 39.2 ºC respectively. The mean weight and age for Kasero *T. parva* positive cattle was 208 kg and 4.5 years respectively. For Kasero *T. parva* negative cattle, the mean weight and age was 231 kg and 4.3 years respectively. The visible mucous membrane observations for cattle that tested positive for *T. parva* in

Kasero were as follows: only 1.8% (1/54) had very pale membranes, 9.2% (5/54) animals were recorded as having pale membranes, while the rest 88.9% (48/54) were considered normal by the examining veterinary official. A total of 24% (13/54) animals were considered sick by their owners while the rest 76% (41/54) were considered healthy even though they were later found to be positive for *T. parva* by PCR.

Only five animals tested positive for *T. parva* by PCR in Makale and they had a mean haemoglobin value of 8.3 g/dl (Table 6. 10). Those animals that tested negative for the same pathogen had a mean haemoglobin value of 10.0 g/dl (Table 6. 11).

Table 6. 10 Clinical parameters of *T. parva* positive cattle in Makale (2006/07 baseline study)

Variable	*N*	*Mean*	*Median*	*StDev*	*Min*	*Max*
Hb (g/dl)	5	8.3	8.6	1.8	5.5	10.2
RT (ºC)	5	38.8	39	0.6	38.2	39.4
Weight (Kg)	5	261	240	59	230	367
Age (Years)	5	6.2	7.0	3.1	3.0	10.0

Hb = Haemoglobin (g/dl), RT = Rectal Temperature (ºC)

Table 6. 11 Clinical parameters for Makale *T. parva* and *T. congolense* negative cattle (2006/07 baseline study)

Variable	*N*	*Mean*	*Median*	*StDev*	*Min*	*Max*
Hb (g/dl)	170	10.0	10.2	2.0	4.7	14.7
RT (ºC)	170	38.9	39.0	0.5	37.7	40.5
Weight (Kg)	170	220	222	100	42	560
Age (Years)	170	4.1	4.0	2.6	0.3	12.

Hb = Haemoglobin (g/dl), RT = Rectal Temperature (ºC)

The difference in mean haemoglobin values between the *T. parva* positive and negative cattle in Makale was found not to be statistically significant (2-sample t-test; estimate for difference = -1.76, 95 % CI [-4.005, 0.482], p = 0.095). The haemoglobin range for *T. parva* positive animals was between 5.5 and 10.2 g/dl while that for the negative animals was from 4.7 to 14.7 g/dl (Figure 6. 7). The animal that had the lowest haemoglobin reading in Makale belonged to the *T. parva* negative group (4.7 g/dl). The *T. parva* negative group also had the animal with the highest recorded haemoglobin value in Makale (14.7 g/dl). The mean rectal temperatures for Makale negative and positive cattle were 38.9 ºC and 38.8 ºC respectively. The mean weight and age for Makale *T. parva* positive cattle was 261 kg and 6.2 years respectively. For Makale *T. parva* negative cattle, the mean weight and age was 220 kg and 4.2 years respectively.

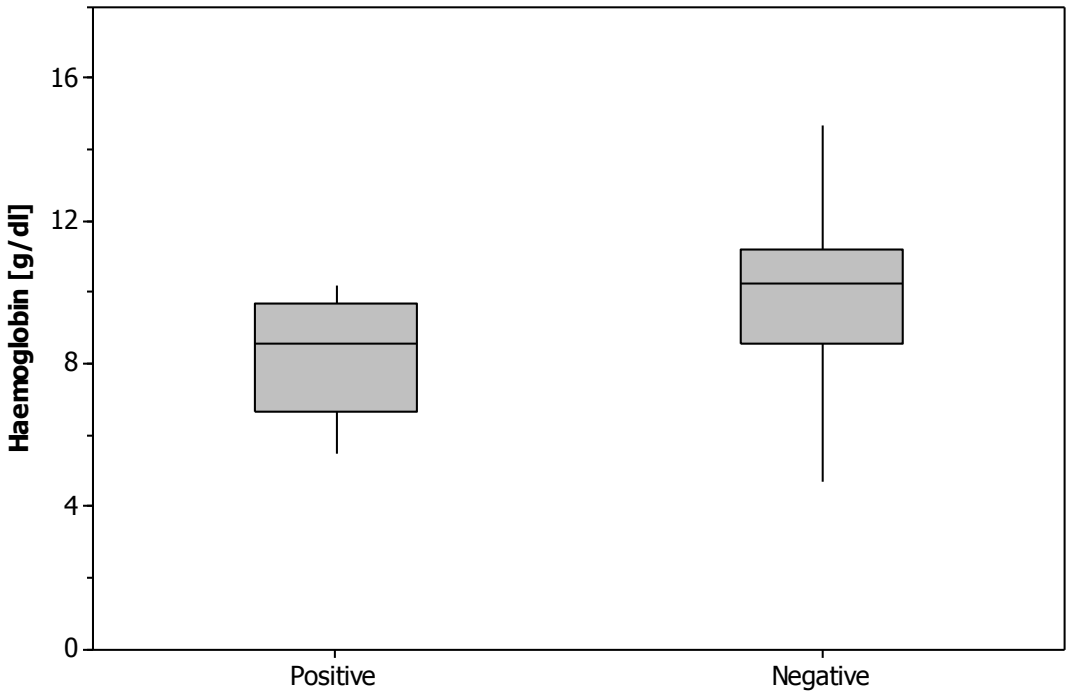

Figure 6. 7 Haemoglobin levels (g/dl) of *T. parva* PCR positive and negative cattle in Makale

Table 6. 11 indicates the haemoglobin and rectal temperature parameters of cattle that tested negative for *theileria parva* and *trypanosoma congolense* in Makale veterinary camp. Makale had a total of 170 cattle (77 %) testing negative against *T. parva* and *T. congolense* at the pre-selection stage while Kasero had 155 cattle (73.5 %) testing negative against the same parasites by PCR at the same study stage.

6.4.3.1.2 Clinical parameters of *T. congolense* positive and negative cattle

There were only three cases of *T. congolense* positive cattle by PCR in Kasero. The first animal had a haemoglobin reading of 9.1 g/dl, rectal temperature of 38.9, weighed 215 kg and was 6 years in age. The second animal had a haemoglobin reading of 10.9 g/dl, rectal temperature of 39.8, and weight of 106 kg and was one year old. The third animal was female, had haemoglobin reading of 6.9 g/dl, was 15 years old, weighed 230 Kg and had a rectal temperature reading of 39.1 ºC. This animal was also found to be positive for *T. parva* by PCR. The difference in mean haemoglobin values between the *T. congolense* positive and negative cattle in Kasero was found to be not statistically significant (2-sample t-test; estimate for difference = -1.7, 95 % CI [-6.71, 3.30], p = 0.28).

Kasero had 155 cattle (73.5 %) testing negative against *T. congolense* and *T. parva* parasites by PCR at the pre-selection stage. Figure 6. 8 is a box and whisker plot of *T. congolense* positive and negative animals in Kasero.

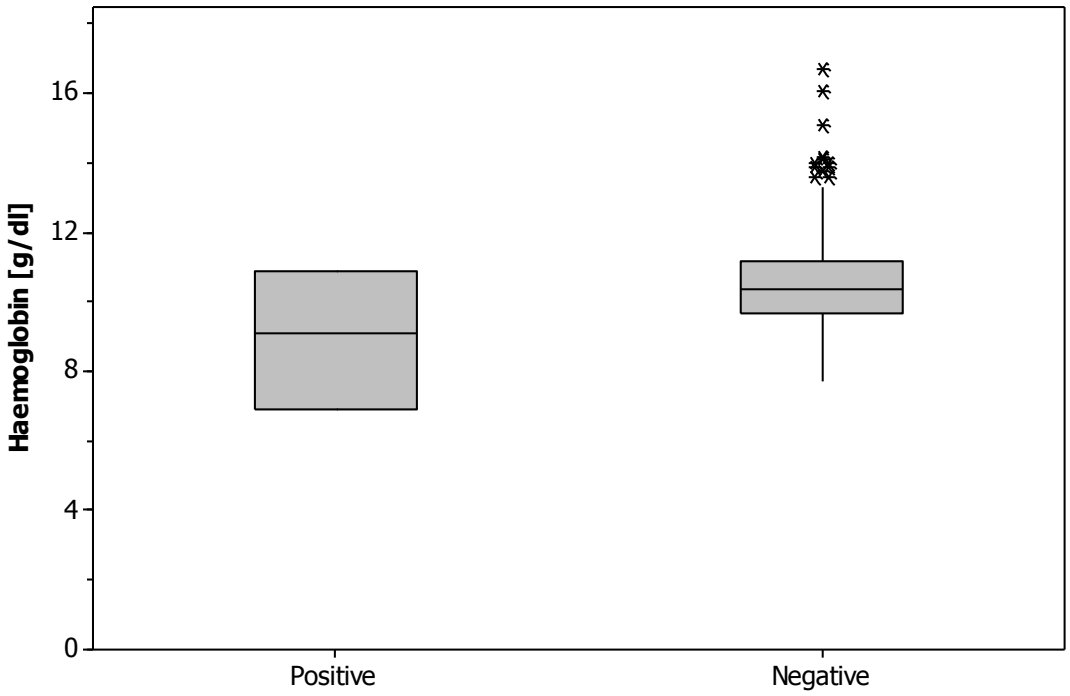

Figure 6. 8 Haemoglobin levels of *T. congolense* PCR positive and negative cattle in Kasero. Note: Because of the nature of the data of positive *T. congolense* cattle in Kasero in which only 3 animals were positive; the plot has no whiskers or outliers.

Animals that tested positive for *T. congolense* by PCR in Makale had a mean haemoglobin value of 7.3 g/dl (Table 6. 12). Those animals that tested negative for the same pathogen had a mean haemoglobin value of 10.0 g/dl (Table 6. 13). The difference in mean haemoglobin values between the *T. congolense* positive and negative cattle was found to be highly statistically significant (2-sample t-test; estimate for difference = -2.73, 95 % CI [-3.35, -2.11], $p < 0.001$).

Table 6. 12 Clinical parameters of *T. congolense* positive cattle in Makale (2006/07 baseline study)

Variable	*N*	*Mean*	*Median*	*StDev*	*Min*	*Max*
Hb (g/dl)	47	7.3	6.9	1.9	4.2	12.2
RT (ºC)	47	38.8	38.8	0.6	37.4	40.5
Weight (Kg)	47	235	241	89	63	475
Age (Years)	47	4.4	4.0	2.6	0.3	10.0

Hb = Haemoglobin (g/dl), RT = Rectal Temperature (ºC)

Table 6. 13 Mean haemoglobin values of T. *parva* and *T. congolense* positive cattle in Kasero and Makale (2006/07 baseline study)

Area	**Pathogen**	**n**	**Mean Hb [g/dl]**	**StDev**	**95 % CI of difference**	**T-value**	**D.F.**	**P-value**	**Sig. diff**
Kasero	Negative	155	10.7	1.5					
	T. parva	54	9.8	1.6	-1.40, -0.39	-3.55	87	<0.001	Yes
	T. congolense	3	9.0	2.0	–6.71, 3.30	-1.46	2	0.281	No
Makale	Negative	170	10.0	2					
	T. parva	5	8.3	1.8	– 4.00, 0.48	-2.18	4	0.095	No
	T. congolense	47	7.3	1.9	–3.35, -2.11	-8.75	77	<0.001	Yes

Sig. diff = Significant difference

The haemoglobin range for *T. congolense* positive animals was between 4.2 and 12.2 g/dl while that for the negative animals was from 4.7 to 14.7 g/dl (Figure 6. 9). The animal that had the lowest haemoglobin reading in Makale belonged to

the positive group (4.2 g/dl). The negative group had the animal with the highest recorded haemoglobin value in Makale (14.7 g/dl).

The visible mucous membrane observations for cattle that tested positive for *T. congolense* in Makale indicated that, 10.6% (5/47) animals had very pale membranes, 19.2% (9/47) animals were recorded as having pale membranes, while the rest 70.2% (33/47) were considered normal by the examining veterinary official. A total of 36.2% (17/47) animals were considered sick by their owners while the rest 63.8% (30/47) were considered healthy even though they were later found to be positive for *T. congolense* by PCR.

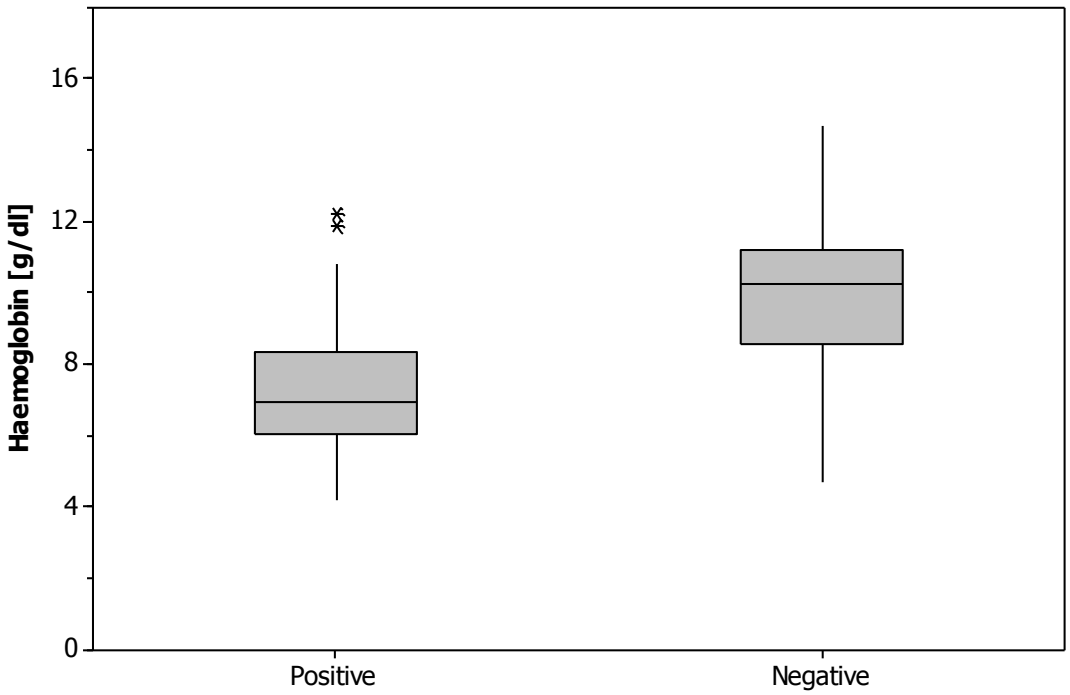

Figure 6. 9 Haemoglobin levels of *T. congolense* PCR positive and negative cattle in Makale

The mean rectal temperatures for Makale negative and positive cattle were 38.9 ºC and 38.8 ºC respectively. The mean weight and age for Makale *T. congolense* positive cattle was 235 kg and 4.4 years respectively. For Makale *T. congolense*

negative cattle, the clinical parameters have already been explained earlier in this chapter (Table 6. 11). Makale had a significant number of animals testing positive for *T. congolense* than Kasero (Table 6. 13).

6.4.3.1.3 Clinical parameters of *T. parva* and *T. congolense* positive cattle

There were two cases of mixed infection by *T. parva* and *T. congolense.* One case was in Kasero and the animal concerned had a low haemoglobin value of 6.9 (g/dl). The animal was female, aged 15 years old, weighed 230 Kg and had a rectal temperature reading of 39.1 ºC. The animal was considered sick by the owner and exhibited pale mucous membranes on examination by the veterinary official. The other case of mixed infection by *T. congolense* and T. *parva* was in Makale. The animal was an 8 year old female and had haemoglobin value of 5.5 g/dl, rectal temperature of 39.3, weighed of 230 kg and was said to be sick by the owner.

6.4.3.1.4 Clinical parameters of cattle that were tested for *T. brucei* and *T. vivax*

There were no cattle that tested positive for *T. brucei* or *T. vivax* by polymerase chain reaction in either Kasero or Makale.

6.4.3.2 Petauke 2008 treatment study

The pre-selection phase of the treatment study had 204 animals sampled in Makale Veterinary Camp. Clinical parameters of these animals at the pre-selection stage are presented in Table 6. 14.

Table 6. 14 Clinical values for Makale Pre-selection Cattle

Variable	*N*	*Mean*	*Median*	*StDev*	*Min*	*Max*
Hb (g/dl)	204	8.9	8.9	2.0	4.6	15.3
RT (ºC)	204	39.3	39.2	0.5	37.0	41.3
Weight (Kg)	204	235	235	87	44	504
Age (Years)	204	3.9	4	2.3	0.1	13

Hb = Haemoglobin (g/dl), RT = Rectal Temperature (ºC)

The mean haemoglobin value for the 204 animals was 8.9 g/dl while that for rectal temperature was 39.3 ºC. The average weight was 235 Kg while the average age was 3.9 years. The range for haemoglobin was between 4.6 g/dl and 15.3 g/dl.

6.4.3.2.1 Pre-treatment stage of treatment study

Table 6. 15 Clinical values for Makale control group

Variable	*N*	*Mean*	*Median*	*StDev*	*Min*	*Max*
Hb (g/dl)	20	6.8	6.9	0.9	4.8	8.2
RT (ºC)	20	39.1	39.1	0.4	38.5	39.7
Age (Years)	20	3.6	4	1.8	1	7

Hb = Haemoglobin (g/dl), RT = Rectal Temperature (ºC)

Table 6. 16 Clinical values for Makale treated group

Variable	*N*	*Mean*	*Median*	*StDev*	*Min*	*Max*
Hb (g/dl)	20	7.0	7.2	0.8	5.2	8.4
RT (ºC)	20	39.0	39.0	0.7	36.5	39.9
Age (Years)	20	3.6	3.5	1.6	1	7

Hb = Haemoglobin (g/dl), RT = Rectal Temperature (ºC)

Forty animals were selected from the initial 204 and paired as explained in Chapter 5 (section 5.3.2). The selected 40 animals were paired and then randomly allocated to either the treatment or control group. Later one motion sensor was attached to each animal in each group. The 40 animals were selected and matched based on the following criteria;

- All animals had haemoglobin levels below 8 g/dl
- All animals in the pairs were as close as possible the same age
- All animals in the pairs were the same sex
- All animals in the pairs were co-grazing and belonging to the same kraal
- Draft animals were excluded from the study
- Animal pairs had as close as possible matching haemoglobin levels at the pre-selection stage

The control group had 20 animals that had mean haemoglobin value of 6.8 g/dl at the pre-treatment stage while the mean rectal temperature was 39.1 ºC (Table 6. 15). The range for haemoglobin was between 4.8 g/dl and 8.2 g/dl.

The treated group had 20 animals that had mean haemoglobin value of 7.0 g/dl at the pre-treatment stage while the mean rectal temperature was 39.0 ºC (Table 6. 16). The range for haemoglobin was between 5.2 g/dl and 8.4 g/dl. There was no significant difference between the mean haemoglobin values for the treated and control groups at the pre-treatment stage (paired t-test; difference of means = 0.2, 95 % CI [- 0.18, 0.60], p = 0.28).

6.4.3.2.2 Clinical values of cattle at the post-treatment stage of treatment study

At the post- treatment stage the mean haemoglobin value for the control group was 7.1 g/dl while the mean rectal temperature was 39.1 ºC (Table 6. 17). The range for haemoglobin was between 4.2 g/dl and 8.4 g/dl.

Table 6. 17 Clinical values for Makale control group (2008 treatment study)

Variable	*N*	*Mean*	*Median*	*StDev*	*Min*	*Max*
Hb (g/dl)	20	7.1	7.4	1.1	4.2	8.4
RT (ºC)	20	39.1	39.0	0.3	38.6	39.8
Age (Years)	20	3.6	4	1.8	1	7

Hb = Haemoglobin (g/dl), RT = Rectal Temperature (ºC)

The treated group had mean haemoglobin value of 10.2 g/dl at the post-treatment stage while the mean rectal temperature was 39.1 ºC (Table 6. 18). The range for haemoglobin was between 8.4 g/dl and 13.5 g/dl. There was a highly significant difference between the mean haemoglobin values for the treated and control groups at the post-treatment stage (paired t-test; difference of means = 3.1, 95 % CI [2.284, 3.946] $p < 0.001$). The discussion of the clinical parameter results in this section is presented at the end of this chapter.

Table 6. 18 Clinical values for Makale treated group (2008 treatment study)

Variable	*N*	*Mean*	*Median*	*StDev*	*Min*	*Max*
Hb (g/dl)	20	10.2	10	1.2	8.4	13.5
RT (ºC)	20	39.1	39.1	0.3	38.6	39.6
Age (Years)	20	3.6	3.5	1.6	1	7

Hb = Haemoglobin (g/dl), RT = Rectal Temperature (ºC)

6.4.4 Relationship of the mean daily number of steps of cattle, rectal temperature, weight and haemoglobin

The relationship between the mean daily number of steps of cattle, rectal temperature, weight and haemoglobin were investigated in the 2008 treatment study (Appendix 8). There was a moderate correlation between the mean number of steps taken by treated cattle in week 2 with the weight of the animals in the Makale treatment study (Pearson's correlation = 0.46, $p < 0.05$) (Appendix 8d). There was no such correlation with the control group of animals (Pearson's correlation = 0.18, $p = 0.46$). Other analysis of combinations of steps and parameters of temperature, weight and haemoglobin did not yield any correlations for the control or treated group of animals (Appendix 8d).

6.5 Discussion

6.5.1 Molecular Parasitology – Kasero and Makale 2006/07

6.5.1.1 *Theileria parva*

There was a marked difference between Makale and Kasero in terms of the proportion of animals that were positive for *T. parva.* Makale only had 2.3 % animals testing positive for *T. parva* while Kasero had 25.6 % animals testing positive for this pathogen. The finding of 25.6 % animals testing positive for *T. parva* in Kasero is slightly lower to that of Mubanga in 2004 who found a prevalence of 32.5% in the same area. The haemoglobin levels of *T. parva* positive animals were only slightly lower than those for *T. parva* negative animals.

Animals in Kasero generally had high haemoglobin values and only 1.9% (4/211) were found to be anaemic (< 8g/dl) by haemoglobinometer. It has been observed that anaemia is not a major diagnostic sign of *T. parva* infection because there is very little division (and therefore destruction) of the parasites in the RBC (Merck Veterinary Manual, 2006; Young, 1988). The difference in mean haemoglobin values between the *T. parva* positive and negative cattle was found to be statistically significant ($p < 0.05$). The higher molecular parasitological proportion of animals with *T. parva* in Kasero was attributed to the higher *theileria parva* challenge in this veterinary camp. During the 2006/07 study, animals that had motion sensors attached were screened for blood on four occasions to test for haemoglobin levels and haemo-parasites. If an animal ever tested positive for *T. parva*, it was again found positive on at least one more occasion later in the study. It is probable that each of the animals found positive was infected throughout the whole period of the study because *T. parva* has been

shown to have a carrier status (Marcotty et al., 2002; Moll et al., 1986). Other than *T. parva*, other species of *theileria* diagnosed in Zambia have included *T. mutans*, *T. velifera* and *T. taurotragi* (Makala et al., 2003).

6.5.1.2 *Trypanosoma congolense*

The number of animals that were positive for *T. congolense* by PCR in Makale was 21.4 % while the number that was positive for *T. congolense* in Kasero was 1.4 %. The finding of 21.4 % animals testing positive for *T. congolense* in Makale is similar to that of Mubanga in 2004 who found a prevalence of 22.5% in the same area. Since cattle management in both areas was similar, the higher molecular parasitological proportion of animals with *T. congolense* in Makale was attributed to the higher *T. congolense* challenge in this veterinary camp. These results confirmed that the northern areas of Petauke district (Makale) have a higher trypanosome challenge than the southern areas (Kasero). These results are in agreement with the surveys conducted between 1982 and 1996 by Berkvens et al that described the *T .parva* vector *Rhipicephalus appendiculatus* being present in the southern parts of Petauke District but slowly spreading northwards mainly through movement of cattle such as work oxen (Berkvens et al., 1998; Billiouw et al., 1999).

The occurrence of *T. congolense* (savannah type) as revealed by the PCR is significant from the veterinary point of view as this type is known to be more virulent than other *T. congolense* types (Bengaly et al., 2002). *T. congolense* is usually very pathogenic and can be looked on as causing a fatal disease in cattle (Taylor, 1930). Makale had a higher proportion of animals with trypanosomiasis and low haemoglobin concentrations and farmers considered trypanosomiasis as

an important disease problem in the area. Makale Veterinary Camp is very near to the South Luangwa National Park in the Luangwa valley. Because of Makale's proximity to the South Luangwa National Park and the high proportion of animals observed with trypanosomiasis, it is likely that the National Park acts as a source of infection for this veterinary camp through its abundant game that act as reservoirs for trypanosomes. Similar results were obtained from previous studies in the area (Machila et al., 2001; Sinyangwe et al., 2004). In East and Central Africa, *T. congolense* has been reported as the most commonly seen trypanosome in wild game species (Uilenberg, 1998). This may have been one of the reasons why this trypanosome was the only species found in Makale. *T. congolense* is also more virulent than *T. brucei* or *T. vivax* (Masumu et al., 2006b) making it more persistent than the other species. Because of its virulence and the chronic nature of the illness it causes, *T. congolense* causes a severe anaemia (Bengaly et al., 2002; Masumu et al., 2006b). It has also been reported that altitude, climate, tsetse density, infection rate, host preference of the tsetse flies and livestock management play an important role in the epidemiology of trypanosomiasis (Connor, 1993; Evison, 1982). It has been shown that there is a higher prevalence of trypanosomiasis in cattle at lower altitudes such as the Luangwa Valley areas in which lie South Luangwa National Park in the Eastern Province of Zambia (Mubanga, 2009). Zebu cattle found in Zambia are extremely susceptible to infections caused by *T. congolense* and *T. vivax* (Billiouw et al., 1999) .

The examining veterinary personnel examined the animals' visible mucous membranes to determine whether they were anaemic (pale) or not. A total of 29.8% of cattle that tested positive for *T. congolense* in Makale were recorded as having pale membranes by examining veterinary personnel. In Kasero, 11% of

cattle positive for *T. parva* were recorded as having pale membranes. The data in the study clearly showed the association between trypanosomiasis and anaemia in cattle, thus demonstrating the usefulness of haemoglobin, anaemia and visible mucous membranes as indicators for disease. Trypanosomiasis is widely known to reduce haemoglobin levels in cattle and low levels have been used in the diagnosis of the disease (Sadun et al., 1973).

6.5.1.3 *Trypanosoma vivax* and *Trypanosoma brucei*

There were no cases of *T. brucei* or *T. vivax* detected by PCR in either Makale or Kasero veterinary camp. Cases of *T. brucei* and *T. vivax* have been reported in the past in the area (Machila et al., 2001; Sinyangwe et al., 2004). Prevalence of *T. congolense* has been reported as 96%, *T. vivax* 2% and *T. brucei* 2% (Sinyangwe et al., 2004). The prevalence in the current study were obtained by PCR as opposed to microscopy which is labour intensive and can lack sensitivity under field conditions because of routine low circulating parasitaemia in infected animals (Picozzi et al., 2002). Taking into consideration the low prevalence of *T. brucei* and *T. vivax,* and that PCR is a much more sensitive method compared to microscopy (Picozzi et al., 2002), it is probable that the animals tested did not have any of the two parasites. The other reason why these two pathogens may not have been detected may have been because farmers in this area have access to trypanocides which they use to treat their animals when they are sick (Chapter 3) and thus clearing off the less virulent and persistent parasites first. Chemotherapy by farmers is also extensively used against trypanosomes in other sub-Saharan countries (Delespaux et al., 2002; Van den Bossche et al., 2000).

6.5.2 Movement activity of cattle that were positive by molecular parasitology

There were four pairs of co-grazing cattle in Kasero that had one animal positive for *T. parva* and the other negative. Three of the animals that were infected took fewer numbers of steps than the non-infected cattle. There was a significant difference in the mean number of steps taken between the infected and non-infected for one of the pairs. Overall, there was no significant difference between the mean numbers of steps of the *T. parva* infected and non-infected cattle in Kasero ($p= 0.21$).

In Makale, there were eight pairs of co-grazing cattle that had one animal positive for *T. congolense* and the other negative. Six of the animals that were infected took fewer numbers of steps than the non-infected cattle. There was a significant difference in the mean number of steps taken between the infected and non-infected for four of the pairs. There was a significant difference between the mean numbers of steps by the *T. congolense* infected and non-infected cattle in Makale ($p< 0.05$).

It has been observed that anaemia is not a major diagnostic sign of *T. parva* infection because there is very little division (and therefore destruction) of the parasites in the RBC (Merck Veterinary Manual, 2006; Young, 1988). *T. congolense* (savannah type) is known to be a more virulent trypanosome than other *T. congolense* types (Bengaly et al., 2002). *T. congolense* is usually very pathogenic and can be looked on as causing a fatal disease in cattle (Taylor, 1930).

Since *T. congolense* infections cause severe anaemia in cattle, there might have been a significant difference in step counts between the infected and non-infected animals with those infected taking fewer steps. This significant reduction of movement activity by cattle that were positive for *T. congolense* might have been because these animals were physically weaker due to the anaemia than their non-infected counterparts. The anaemia meant a reduction in haemoglobin and therefore in the oxygen-carrying capacity of the blood. When insufficient oxygen is available to the cells for their efficient functioning, the efficiency of their normal activities is reduced which leads to a slow process of deterioration of health and condition of the animal (Uilenberg, 1998).

6.5.3 Clinical profiles of cattle in 2006/07 baseline and 2008 treatment studies

In this chapter, the clinical parameter profiles of over 600 cattle were determined in pre-selection screening in the two veterinary camps of Kasero and Makale during the 2006/07 baseline study and in Makale during the 2008 treatment study.

6.5.3.1 Clinical parameters and molecular parasitology of cattle in Baseline study (2006/07)

The mean haemoglobin concentration observed in Makale was lower than that which was observed in Kasero. The difference in mean haemoglobin values recorded for cattle sampled in Makale and Kasero during the baseline study was found to be significant. Animals in Kasero generally had higher haemoglobin values and only 1.9% (4/211) were found to be anaemic (< 8g/dl) by haemoglobinometer. In Makale, a total of 28.5% (63/221) of animals were found

to be anaemic by haemoglobinometer. The significant difference in the haemoglobin values observed for Kasero cattle and Makale cattle makes it imperative to consider haemoglobin values when evaluating animal health in an area. The reason for this difference in haemoglobin values was most likely due to the different diseases profiles of the two areas.

These two areas showed a difference in the proportion of cattle that were positive for *T. parva* and *T. congolense.* It has been shown that trypanosomiasis (Akinbamijo et al., 1998; Cherenet et al., 2006; Losos and Ikede, 1972) and more specifically *T. congolense* can cause severe anaemia in cattle (Bengaly et al., 2002; Masumu et al., 2006a).

6.5.3.2 Clinical parameters of cattle in 2008 treatment study

The 204 cattle that were sampled in the pre-selection stage of the study gave an overview of the clinical picture obtaining in Makale veterinary camp prior to commencement of the 2008 treatment study.

The clinical data that was obtained at the pre-treatment stage provided information on the clinical picture of the control group of animals and those that were in the treated group one week before treatment was administered. It was found that there was no significant difference between the mean haemoglobin values for the treated group and control group. This was expected as the animals were coming from the same area under similar disease challenges and management.

The post-treatment stage of the experiment revealed a highly significant difference between the mean haemoglobin values for the treated group and control groups. There was a 3.2 g/dl increase in mean haemoglobin concentration of the treated group from 7.0 g/dl at the pre-treatment stage to 10.2 g/dl at the end of the experiment. The group shifted from being anaemic to having normal haemoglobin concentration of above 8 g/dl (Schalm et al., 1975). The control group saw a slight increase of 0.3 g/dl in mean haemoglobin concentration from 6.8 g/dl to 7.1 g/dl but still remained anaemic (< 8 g/dl). The results showed that the drugs that are commonly used in Petauke district to treat trypanosomiasis, East Coast Fever and pasture transmitted diseases do have a positive effect on increasing the cattle haemoglobin concentrations. Drugs such as antibiotics, anthelmintics and trypanocidal drugs are readily available at the Petauke District Veterinary Office for farmers to buy when they need them. It has been demonstrated that even though there is some drug resistance against diminazene aceturate (Sinyangwe et al., 2004), this drug is still an effective treatment against trypanosomiasis in Petauke District.

CHAPTER 7

General Discussion

7.1 Overview

Most of the people in Petauke District of the Eastern Province of Zambia are traditional subsistence farmers practicing a mixed crop and livestock production farming system. They rely on cattle as a source of draft power critical for crop cultivation and transportation. Cattle are their main assets and provide other benefits such as manure for improving soil fertility, meat, milk and eggs for consumption or sale (Anteneh Addis 1987 ; Perry et al., 2002).

Endemic livestock diseases such as trypanosomiasis, ECF, babesiosis, anaplasmosis, cowdriosis and helminthic infections continue to hamper livestock production among farmers in sub-Saharan Africa. All these diseases have anaemia as an important presenting clinical sign (Bundza and Samagh, 1982; Eisler et al., 2004; Hendrickx et al., 2004; McCrorie et al., 1980; Minjauw and Mcleod, 2003). Using haemoglobin as an objectively verifiable indicator of disease, the movement behaviour of cattle were investigated using IceTag™ two-dimensional motion sensors in Makale and Kasero Veterinary Camps of Petauke District. The work presented in this thesis investigated the suitability of using two-dimensional motion sensors in a traditional African farming system during the rainy season. Movement behaviour patterns of cattle in two veterinary camps within the district were investigated. The thesis further investigated the impact of a triple co-administered broad-spectrum treatment on cattle movement behaviour. Other aspects investigated were the prevalence of trypanosomiasis and ECF in the two veterinary camps where the study was conducted and also the cattle management practices of the farmers in the area.

7.2 Summary of findings

7.2.1 Suitability of motion sensors as tools for studying cattle movement

The results of this study clearly showed that two-dimensional motion sensors could be used to study cattle movement behaviour in a traditional African livestock management system. The technology was accepted by both the farmers and their animals. The farmers were very co-operative throughout the whole programme as they understood that the long term goals of the study would benefit their community. The farmers accepted the technology to be used on their animals as they were fully informed of what was happening from the beginning until the end of the study. It has been observed that when people understand a control option and its potential benefits, they are likely to respond positively (Tatchell, 1981). Cattle also appeared not to be too bothered by the attachment of the motion sensors to their hind legs. As a precaution of preventing too much discomfort due to attached sensors, the Velcro straps attaching the units to the animals were not attached too tightly around the legs. Additionally, the sensors were changed to the other hind leg after a week of attachment to further reduce discomfort due to prolonged attachment. The sensors could be easily attached and removed from a well restrained animal with minimal effort using the Velcro strap attachments. The motion sensors successfully recorded the movement behaviour of standing, walking and lying down during the duration of attachment to the animals with minimal labour. It has been previously observed that sampling cattle behaviours demands a high degree of labour, equipment, and time (Mitlohner et al., 2001; Robert et al., 2009).

7.2.2 Cattle movement behaviour in Petauke District (2006/07 study)

Two-dimensional motion sensors successfully recorded the step counts, proportion of time spent standing, walking and lying down in Petauke District of the Eastern Province. Motion sensors have been evaluated to monitor and classify animal behaviour successfully by other researchers (Robert et al., 2009; Walker et al., 1985). Cattle in Kasero were found to have significantly ($p < 0.05$) higher mean step counts than Makale cattle. Kasero animals were also found to have significantly higher haemoglobin values than Makale cattle ($p < 0.05$). It is suggested that Makale animals had lower haemoglobin levels because of the higher prevalence in this area of a pathogen known to cause anaemia (*T. congolense*, savannah) (Bengaly et al., 2002; Masumu et al., 2006c; Taylor, 1930).

In Makale, there was a significant difference between step counts of the high and low haemoglobin groups of cattle ($p < 0.05$). There was also a significant difference between the mean haemoglobin values of the high and low groups ($p < 0.05$). Low haemoglobin cattle took fewer steps than high haemoglobin cattle. Makale had a larger proportion of animals having haemoglobin values below 8g/dl. As a result, when selecting animals into the high and low haemoglobin groups, it was easier to find animals at both ends of the scale while still fulfilling the other selection criteria. Because the two groups were distinctly divided on the basis of circulating haemoglobin levels, this might have led to the difference in movement behaviour with high haemoglobin animals having higher step counts than those in the low group with haemoglobin values below 8g/dl.

Kasero cattle that had motion sensors attached showed no significant difference of step counts between the high and low haemoglobin groups ($p = 0.75$). This is

despite there being a significant ($p < 0.05$) difference in the mean haemoglobin values between the two groups. This may have been because both the low and high haemoglobin groups in Kasero had mean haemoglobin levels above what are considered normal levels (Schalm et al., 1975) of 8g/dl. This may have resulted in all cattle in the area behaving in a normal manner in terms of their movement patterns and showing no differences between the two groups because they all had above normal haemoglobin levels.

There was a significant difference between Makale and Kasero mean step counts ($p < 0.05$). Kasero cattle had higher mean step counts, higher mean haemoglobin values and higher proportion of animals infected with *T. parva*. Makale animals had lower mean step counts, lower mean haemoglobin values and a higher proportion of the animals were infected with *T. congolense*. The *T. congolense* infections may have been the cause of the low haemoglobin values in cattle in this area resulting in the possible reduction of movement activity as cattle might have been physically weaker due to the anaemia caused by this parasite than their counterparts in Kasero. The anaemia meant a reduction in haemoglobin and therefore in the oxygen-carrying capacity of the blood. When insufficient oxygen is available to the cells for their efficient functioning, the efficiency of their normal activities is reduced which leads to a slow process of deterioration of health and condition of the animal (Uilenberg, 1998).

A Principal Components Analysis (PCA) was used in this study to investigate which of the three variables (standing, walking or lying down) was more important in determining the overall variability of the motion sensor data over a twenty four hour period. Score plots from the PCA of Makale and Kasero cattle revealed separate clusters of cattle from the two areas reflecting underlying

differences between their movement patterns. The PCA indicated that clustering was mainly based on the differences in night time standing and lying behaviour between the two areas. The PCA and cattle movement behaviour profiles revealed that cattle in Kasero spent more time lying down than Makale cattle between the hours of 21:00 hours at night and 06:00 hours in the morning. Conversely, Makale cattle spent more time standing than Kasero cattle between the same hours. These results indicated that the two study sites differed in terms of their cattle night time standing and lying behaviour. Cattle lying behaviour has been used as a measure of cattle well-being in the dairy industry (Cook et al., 2005; Robert et al., 2009). The differences in night time lying and standing behaviour might also have been due to the conditions of kraals where the animals slept during the study period. Due to limitations on the number of motion sensors available, the study was initially carried out in Kasero before being repeated in Makale. The rains had started during the study in Kasero but intensified as the study moved to Makale (author's personal observation). This difference in time (three weeks) of carrying out the study meant that by the time the experiment was carried out in Makale, the rains were heavier which might have explained why animals were standing more because the ground was wet and muddy making it less appealing for lying down.

In the dairy industry, differences in standing activity have been associated with lameness in cattle (Cook et al., 2005). Farmers in both Kasero and Makale reported foot-rot (interdigital phlegmon, foul in the foot) as one of the important cattle diseases in the area (Chapter 3, Section 3.3.3). Foot-rot is associated with lameness in cattle (Merck Veterinary Manual, 2006) and may explain why cattle in one area spent more time standing up than in another area. Large increases in standing time have been observed with increasing severity of lameness (Cook et

al., 2005). As Makale cattle spent more time standing, this may have been an indication that they might have had a higher proportion of lame animals although this was not observed in the field by the researchers. A further benefit of cows spending time lying down is the effect on rumination. Cows spend more time ruminating while lying down than standing up. If cows spend more time ruminating they may produce more saliva which leads to a reduction of rumen acidity which can help to alleviate problems such as acidosis and laminitis especially in dairy animals (Blackie et al., 2006).

The walking behaviour profile for both areas was not very different. Animals in both areas started the day at around 07:00hrs and stopped almost all walking activity by 21:00hrs. Overall, there was a slight decrease in walking behaviour between 14:00hrs and 15:00hrs. This corresponded with a slight increase in lying behaviour around the same period. This appeared to be an indication that animals stopped grazing and were resting at this time of day. Animals around this time of the day were seen resting under the shades of trees as it was very hot (author's personal observation). The time before and after the period between 14:00hrs and 15:00hrs was spent mostly in activity either standing or walking.

7.2.3 Movement behaviour of cattle that were given a broad-spectrum treatment

The results in this study showed that a three co-administered broad-spectrum drug treatment improved low circulating haemoglobin levels in cattle to what are considered normal levels. Cattle that were in the treated group had significantly improved haemoglobin levels ($p<0.001$) compared to control cattle ($p=0.13$). Improvement of circulating haemoglobin levels and condition have been demonstrated following treatment of animals by diminazene aceturate (Biryomumaisho, 2007; Van den Bossche et al., 2000). The treated group's mean haemoglobin profile improved from 6.94 g/dl at the pre-treatment stage to 8.14 g/dl at the post-treatment stage. The control group's mean haemoglobin levels remained below 8 g/dl both at the pre-treatment stage (6.87 g/dl) and post-treatment stage (7.14 g/dl). This meant that cattle in the control group remained below the 8 g/dl threshold and were considered anaemic (Schalm et al., 1975).

The movement behavioural patterns of cattle in the study were observed one week before and two weeks after treatment. The drugs commonly used to treat against disease pathogens present in the area were diminazene aceturate, long-acting oxytetracycline and the broad spectrum anthelmintic, albendazole. This reflected the normal management practices in the area and the drugs are used to treat trypanosomiasis, tick borne diseases and pasture-transmitted diseases. The numbers of steps taken by cattle were one of the parameters used to study behavioural patterns. There were no significant differences between the mean numbers of steps of control and treated groups of cattle at the pre-treatment stage. This was as expected because animals were from the same area and under a similar cattle management system. Additionally, the animals faced the same disease challenges in the area and this was reflected by similar haemoglobin

values at the pre-selection stage. At the post-treatment stage of the experiment, the treated group had significantly higher step counts than the control group two weeks after treatment ($p<0.05$). The difference in haemoglobin levels mentioned earlier might have contributed to enabling the treated group to maintain higher step counts while the control group of animals suffered the effects of lower circulating haemoglobin levels.

Treated cattle showed a significant difference between the number of steps taken from the week before treatment to the second week after treatment ($p<0.05$). This group saw their mean haemoglobin levels improve probably due to the drug administration. The improved haemoglobin levels may have aided the animals in the treated group have mean step counts rise from 6,697 steps per day one week before treatment to 8,131 steps per day two weeks after treatment. Significant numbers of steps were noticed for cattle in the treated group during the second week possibly because this is when the effects of the administered drugs were being physically manifested. Treated cattle showed a 17% increase in step counts during the second week compared to a 2.6% increase by control cattle. During the second week after treatment, not all treated animals responded by having increased step counts. It is possible that some animals may not have responded to treatment after two weeks because they needed more time to respond or possibly because of drug resistance especially against diminazene aceturate. It has been shown that there has been a five-fold increase in *T. congolense* isolates resistant to diminazene aceturate in the Eastern Province of Zambia from 1996 to 2003 (Delespaux et al., 2008). Since there were no indications that the drug pressure increased between 1996 and 2003, it was suggested that genetic exchange of resistance genes might explain the increased frequency of resistance to diminazene aceturate (Delespaux et al., 2008).

It would have been interesting to see how long animals with increased step counts would have sustained them if the experiment had carried on longer than the two week period. It would also have been interesting to see if all treated animals would have increased their step counts if they had been monitored for a longer period. The results showed that there were no differences in the mean number of steps taken between the treated and control groups of cattle at the start of the study ($p=0.71$) in the week before treatment. There was also no significant difference between the number of steps taken from the week before treatment to the first week after treatment for both treated ($p=0.36$) and control ($p=0.45$) cattle. However, in the week following treatment, control cattle took significantly more steps than treated cattle ($p<0.05$). This could have been because this is when the treated animals were starting to respond to the treatment. The treated animals' physiology may have been affected after drug administration and this may have negatively affected their step counts as they went through the convalescence period.

Mean cattle time budgets in Petauke District revealed the percentage of time cattle spent standing, walking or lying down during the whole duration of the treatment study. At the pre-treatment stage, the time budgets revealed that in a twenty four hour period, cattle in the treated group spent 49% of their time standing and 40% lying down. The remainder of the time (11%) was spent walking. Cattle in the control group spent 50% standing, 38% lying down and 12% walking. At the post-treatment stage during week one, control cattle spent more time walking than cattle in the treated group. In the second week post-treatment, treated cattle spent more time walking ($p < 0.05$) and lying down ($p <$

0.001) than control cattle. On the other hand, control cattle spent more time standing during the week than those in the treated group ($p < 0.001$).

A principal components analysis was also used to further analyse the data. The PCA revealed that there were differences in the behaviour of treated and control cattle based on their movement patterns. The score plots from the PCA revealed two distinct clusters within the treated group (Figures 17 and 18) while the control group cattle were evenly distributed and showed no distinct clusters. This pattern was a reflection of the underlying differences in behaviour between the treated cattle and those in the control group. The PCA showed that the greatest difference between the behaviours of the two groups was in their standing and lying behaviour. Animals that were treated and ended up having higher haemoglobin levels were found to be closer together on the score plots. This may be because they exhibited "normal" behaviour while the control cattle that had lower haemoglobin levels (and therefore might have been sick animals) were more scattered on the score plots implying the more erratic behaviour of weak or stressed animals. It has been shown that the level of anaemia, haemoglobin or the PCV usually gives a reliable indication of the disease status and productive performance of an infected animal (Trail et al., 1991). Cattle lying behaviour has been used as a measure of cattle well-being in the dairy industry (Cook et al., 2005; Robert et al., 2009). The PCA appears to have teased out the qualitative and quantitative distinctions in the data and suggested that administering a broad spectrum of drugs reduced variability among cattle in terms of movement and that the reduction in variability related to movement behaviour at particular times of the day.

These investigations have confirmed that motion sensors may be used to investigate movement behaviour in traditionally managed cattle, and that whereas reduced haemoglobin levels associated with various parasitic infections may suppress cattle movement activity, treating these infections and improving haemoglobin levels may improve or restore more normal movement behaviour patterns. The results have shown that the treatment given was effective and improved circulating haemoglobin levels. It has been demonstrated that even though there is some drug resistance against diminazene aceturate, this drug is still an effective treatment against trypanosomiasis in Petauke District (Sinyangwe et al., 2004). Diminazene aceturate is also known to be effective against babesiosis. The advent of safe and effective broad-spectrum anthelmintics has largely reduced the need for differentiating the genera and species of helminths (Merck Veterinary Manual, 2006).

7.2.4 Trypanosomiasis and ECF in Kasero and Makale Veterinary Camps

There was a marked difference between Makale and Kasero in terms of the proportion of animals that were positive for *T. parva.* Makale only had 2.3 % animals testing positive for *T. parva* while Kasero had 25.6 % testing positive for the same pathogen. The haemoglobin levels of *T. parva* positive animals were only slightly lower than those for *T. parva* negative animals. Animals in Kasero generally had high haemoglobin values and only 1.9% (4/211) were found to be anaemic (< 8g/dl) by haemoglobinometer. It has been observed that anaemia is not a major diagnostic sign of *T. parva* infection because there is very little division (and therefore destruction) of the parasites in the RBC (Merck Veterinary Manual, 2006; Young, 1988). The higher molecular parasitological proportion of animals with *T. parva* in Kasero was attributed to the higher *T.*

parva challenge in this veterinary camp. During the 2006/07 study, animals that had motion sensor attached were sampled for blood on four occasions to test for haemoglobin levels and haemo-parasites. If an animal ever tested positive for *T. parva*, it was again found positive on at least one more occasion later in the study. It is probable that each of the animals found positive was infected throughout the whole period of the study because *T. parva* has been shown to have a carrier status (Marcotty et al., 2002; Moll et al., 1986). Other than *T. parva*, other species of *theileria* diagnosed in Zambia have included *T. mutans*, *T. velifera* and *T. taurotragi* (Makala et al., 2003).

The number of animals that were positive for *T. congolense* by PCR in Makale was 21.4 % while the number that was positive for the same pathogen in Kasero was 1.4 %. Since cattle management in both areas was similar, the higher molecular parasitological proportion of animals with *T. congolense* in Makale was attributed to the higher challenge by this pathogen in this veterinary camp.

These results confirmed that the northern areas of Petauke district (Makale) have a higher trypanosome challenge than the southern areas (Kasero). Conversely, the southern areas of the district were found to have a higher *T. parva* challenge than the northern areas. These results are in agreement with the surveys conducted between 1982 and 1996 by Berkvens et al that described the *T .parva* vector *Rhipicephalus appendiculatus* being present in the southern parts of Petauke District but slowly spreading northwards mainly through movement of cattle such as work oxen (Berkvens et al., 1998; Billiouw et al., 1999).

The occurrence of *T. congolense* (savannah type) in Makale as revealed by the PCR is significant from the veterinary point of view as this type is known to be

more virulent than other *T. congolense* types (Bengaly et al., 2002). *T. congolense* is usually very pathogenic and can be looked on as causing a fatal disease in cattle (Taylor, 1930). Makale had a higher proportion of animals with trypanosomiasis and anaemia and therefore farmers considered trypanosomiasis as an important disease problem in the area**.**

Makale Veterinary Camp is very near to the South Luangwa National Park in the Luangwa valley. Because of Makale's proximity to the South Luangwa National Park and the high proportion of animals observed with trypanosomiasis, it is likely that the National Park acts as a source of infection for this veterinary camp through its abundant game that act as reservoirs for trypanosomes. Similar results were obtained from previous studies in the area (Machila et al., 2001; Sinyangwe et al., 2004). In East and Central Africa, *T. congolense* has been reported as the most commonly seen trypanosome in wild game species (Uilenberg, 1998). This may have been one of the reasons why this trypanosome was the only one found in Makale. *T. congolense* is also more virulent than *T. brucei* or *T. vivax* (Masumu et al., 2006b) making it more persistent than the other species. Because of its virulence and the chronic nature of the illness it causes, *T. congolense* causes a severe anaemia (Bengaly et al., 2002; Masumu et al., 2006b). It is also known that voluntary feed intake by cattle during trypanosomiasis infections may be depressed, and this may compound the effects of the trypanosome infection on the haematological parameters of the host (Akinbamijo et al., 1998; Losos and Ikede, 1972). It has been reported that altitude, climate, tsetse density, infection rate, host preference of the tsetse flies and livestock management play an important role in the epidemiology of trypanosomiasis (Connor, 1993; Evison, 1982). It has been shown that there is a higher prevalence of trypanosomiasis in cattle at lower altitudes such as the Luangwa Valley areas

that contain South Luangwa National Park in the Eastern Province of Zambia (Mubanga, 2009). As other diseases other than trypanosomiasis may also cause anaemia, anaemia may be used as a measure of the general health status when surveying herd health (Hendrickx et al., 2004).

There were no cases of *T. brucei* or *T. vivax* detected by PCR in either Makale or Kasero veterinary camp. Cases of *T. brucei* and *T. vivax* have been reported in the past in the area (Machila et al., 2001; Sinyangwe et al., 2004). Prevalence of *T. congolense* has been reported as 96%, *T. vivax* 2% and *T. brucei* 2% (Sinyangwe et al., 2004). The prevalence in the current study were obtained by PCR as opposed to microscopy which is labour intensive and can lack sensitivity under field conditions because of routine low circulating parasitaemia in infected animals (Picozzi et al., 2002). Taking into consideration the low prevalence of *T. brucei* and *T. vivax,* and that PCR is a much more sensitive method compared to microscopy (Picozzi et al., 2002), it is probable that animals tested were not infected by any of the two parasites. The other reason why these two pathogens may not have been detected may have been because farmers in this area have access to trypanocides which they use to treat their animals when they are sick (Chapter 3) and thus clearing off the less virulent and persistent parasites first. Chemotherapy by farmers is also extensively used against trypanosomes in other sub-Saharan countries (Delespaux et al., 2002; Van den Bossche et al., 2000).

Robinson et al (2002) noted that in spite of the importance of trypanosomiasis in Zambia, economic instability and donor fatigue have led to a shortage of operational funds within the Zambian government veterinary department, resulting in a rapid decline of resources available for tsetse and trypanosomiasis control (Van den Bossche et al., 2000). This, combined with a relative lack of

success in 'area-wide' control, has resulted in a change in emphasis from widespread eradication towards smaller-scale, community-based interventions that require disease management rather than purely vector control. It is, therefore, increasingly important to identify areas that are of high priority for control (Robinson et al., 2002).

7.2.5 Clinical parameters and molecular parasitology of cattle in 2006/07 and 2008 studies

The mean haemoglobin concentration observed in Makale was lower than that which was observed in Kasero. The difference in mean haemoglobin values recorded for cattle sampled in Makale and Kasero during the 2006/07 motion sensor study was found to be significant ($p < 0.01$). Animals in Kasero generally had higher haemoglobin values and only 1.9% (4/211) were found to be anaemic (< 8g/dl) by haemoglobinometer. In Makale, a total of 28.5% (63/221) of animals were found to be anaemic by haemoglobinometer. The significant difference in the haemoglobin values observed for Kasero and Makale cattle makes it imperative to consider haemoglobin values when evaluating animal health in an area. The reason for this difference in haemoglobin values was most likely due to the different disease profiles of the two areas.

During the study, veterinary field officers examined the visible mucous membranes of cattle that had tested positive for *T. congolense* and determined that 29.8% had pale membranes. This value closely matched the anaemia percentage obtained by haemoglobinometer of 28.5%. In Kasero, 11% of animals were determined as having pale membranes. This percentage was higher than that obtained by haemoglobinometer (1.9%). The data in the study clearly showed the association between trypanosomiasis and anaemia in cattle, thus

demonstrating the usefulness of haemoglobin and anaemia as an indicator for disease. Trypanosomiasis is widely known to reduce haemoglobin levels in cattle and low levels have been used in the diagnosis of the disease (Sadun et al., 1973).

The clinical data that was obtained in the 2008 treatment study at the pre-treatment stage provided information on the clinical picture of the control group of animals and those that were in the treated group one week before treatment was administered. It was found that there was no significant difference between the mean haemoglobin values between the two groups. The post-treatment stage of the experiment revealed a highly significant difference between the mean haemoglobin values for the treated group and control groups. There was a 3.2 g/dl increase in mean haemoglobin concentration of the treated group from 7.0 g/dl at the pre-treatment stage to 10.2 g/dl at the end of the experiment. The group shifted from being anaemic (<8g/dl) (Schalm et al., 1975) to having haemoglobin concentrations of above 8 g/dl. The control group saw a slight increase of 0.3 g/dl in mean haemoglobin concentration from 6.8 g/dl to 7.1 g/dl but still remained anaemic (< 8 g/dl). The results showed that the drugs that are commonly used in Petauke district to treat trypanosomiasis, tick borne diseases and pasture transmitted diseases do have a positive effect on increasing the cattle haemoglobin concentrations.

7.2.7 Cattle management practices in Petauke District

A majority of cattle raising households in Petauke district employ herders to herd their animals seven days a week mainly during the rainy season when there are crops to protect. Niamir reported similar findings among the Ngoni people of Chipata town (formerly Fort Jameson) in Eastern Province of Zambia (Niamir, 1990). The majority of the cattle herders do not go to school and are not related to the cattle owner but are employees. The cattle owner provides the herders accommodation and food and after a period of four to five years they are paid a female animal and this signifies the end of the contract term for herding cattle.

In both Kasero and Makale, the number of households raising cattle, pigs and goats was higher than the number raising cattle and pigs or cattle and goats combinations. Having other types of livestock like pigs and goats enables the farmer to have a buffer in times of difficulties. He can sell off the cheaper animals first should he need urgent money before resorting to selling cattle which are more expensive and are his most valuable asset. Even though each household has their own herder, the livestock of the village often meet and mix in the communal grazing areas (Chapter 3, personal observation). The grazing areas are natural pastures which are the cattle's main food supply. After the farmers harvest their crops from around May, cattle have access to crop residues of mainly maize and groundnuts. When these residues run out, there is a problem of grazing and farmers have to move further from the villages to feed their animals (Chapter 3). It is probable that in Makale Veterinary Camp, as herders search for grazing areas for their cattle, they move closer to the Kafue National Park which has abundant game that act as reservoirs for trypanosomes. They

may therefore put their animals at greater risk of trypanosomiasis during this period.

All animals in the study area were grazed for an average of nine hours during the day and kept in kraals during the night. Kraaling is also practised in other Southern African countries and is a long-established custom and is regarded as the right thing to do by many cattle owners as it is supposed to be a protective measure against wild predators (Reed et al., 1974). In Petauke District, kraaling is also practiced to prevent cattle rustling. The kraals in the study area were in all instances made of logs forming a circular fence and were not roofed. The size of the kraal depended on the size of the herd with larger herds having larger kraals. In most cases the farmer built his kraal in an area which would include an anthill to provide raised ground. This would provide an area of high dry ground for animals in the rainy season. It was found that dry ground provided by the anthills in the kraals was in most cases inadequate and lead to a lot of animals standing on lower ground which was poorly drained because of the dung and wet mud (personal observation). The kraals where cattle are kept at night play an important role in the management of cattle. The cattle are kept in kraals for at least 12 hours and during the rainy season the ground becomes heavily contaminated with dung and mud making ideal conditions for transmission of disease pathogens (Kaufmann et al., 1993). This may be the reason that footrot was reported as an important disease in both the study areas. It has been shown that frequent changing of the cattle holding site reduces the risk as measured by disease pathogen densities in the soil and increased the weight gain of the animals (Kaufmann et al., 1993). This practice of frequent changing of kraals during the rainy season was not described by farmers or observed by the researcher in Petauke District during the study period.

Cattle diseases, lack of water and grazing land shortage were cited as cattle production constraints in both Kasero and Makale. Previous studies have shown that larger grazing areas cause more excessive walking in animals, while time spent walking decreases in correspondence with grass availability (Arave C. W., 1981). Therefore, during periods of reduced availability of grazing areas, cattle in both areas would be expected to have greater movement activity.

The office of the District Veterinary Office has reported in the past (DVO, 2008) that farmers in most cases buy therapeutic drugs or report animals that are sick to the veterinary office when diseases are already advanced and it's too late to save the animal. A majority of livestock owners rely on their herd boys to tell them when the animals are sick. The average age of herd boys for both areas was 11.2 years but they could be as young as 4 or 5 years old. The herd boys are young people who may not be able to notice sick animals in the very early stages of illness. Furthermore, the herd boys are not included in or invited to farmer training sessions organized by the veterinary department and livestock non-governmental organisations (NGOs) and yet are expected to first notice and tell the farmer when the animal is sick. A change in the target groups of extension messages from the veterinary department and NGO's to include herd boys may be warranted.

7.3 Motion sensor practical considerations

The operational performance of the motion sensors was found to be very satisfactory. After one week of attachment, the motion sensors had data downloaded and were changed to the other hind leg to as much as possible reduce discomfort to the animal and avoid losing all data in the case of loss of a unit. The weekly process of removal and reattachment of Velcro-straps had budgetary implications. This was because mud and manure accumulation on the used straps caused the straps to incompletely attach after use and necessitated using a new strap in most cases. In only one instance did a motion sensor fall from the animal's leg before the next scheduled data download and that resulted in one week's data loss during the 2008 study. The motion sensors appeared to function properly on either hind leg of the animal. The motion sensor cross validation design of the 2006/07 study of exchanging sensor units between animals in the pairs after one week of attachment did not detect any abnormally functioning units. The amount of activity also did not change when a motion sensor was switched from the right hind leg to the left hind leg or vice versa in the 2008 treatment study. The caps on the motion sensors that covered the USB connection points on a number of occasions came off during attachment. This resulted in data not being able to be immediately downloaded because the contact points were full of mud. Data were successfully downloaded after removing the mud using a sharp pin. The problem was overcome by covering the caps with masking tape to hold them in place.

IceTag™ two-dimensional motion sensors successfully monitored and quantified cattle movement behavioural characteristics in a traditionally managed crop-

livestock farming system in the Eastern Province of Zambia. Despite being deployed during adverse wet and muddy weather conditions in the rainy season, the motion sensors automatically collected data on the standing, walking and lying behaviour of large numbers of cattle with very minimal labour and caused no major technical problems during the data capture phase. The use of motion sensor technology was successfully used in combination with clinical and molecular methods to provide a starting point for understanding movement behaviour of traditionally managed cattle in the context of improving animal health and production in rural sub-Saharan Africa. The usefulness of motion sensors as a potential tool for the monitoring and evaluation of cattle movement behaviour was highlighted by the results of the study.

Through molecular parasitological techniques, the two veterinary camps of Makale and Kasero were identified as areas where trypanosomiasis and East Coast Fever respectively constitute major animal health problems. A structured questionnaire on livestock ownership and management practices also showed that cattle owners considered trypanosomiasis and theileriosis the main constraints to improved cattle health and production in their traditional crop-livestock mixed farming system. There were significant differences in haemoglobin levels between the two camps with Makale having lower levels than Kasero. Baseline data indicated that a larger proportion of sampled animals in Makale had trypanosomiasis while those in Kasero had theileriosis. Low circulating haemoglobin levels were linked to trypanosomiasis prevalence.

Analysing the motion sensor data from the three week treatment study in Makale revealed that the treated animals (which had higher mean haemoglobin values at the end of the study) were clustered more closely than the control

animals (which had lower mean haemoglobin values). The numbers of steps taken by high haemoglobin cattle in both studies were significantly higher than the low haemoglobin cattle at the end of the experiments. This, coupled with the PCA results suggests an association between cattle haemoglobin levels and their movement patterns. This study provided a baseline for future research on traditionally managed cattle movement behaviour in rural sub-Saharan Africa.

7.5 Future work

- Both motion sensor studies described in this thesis were carried out during the rainy season (November to April). During this period, both water and grazing are abundant and animals do not have to travel great distances to drink or graze. In the dry season animals travel further for feed and water. It would be interesting to compare dry season (May to October) movement patterns (when there is scarce food and water) with those of the rainy season.
- In the 2008 study, animals in the treated group had increased step counts. A study longer than three weeks would help determine how long animals with increased steps counts can sustain them. Increasing the duration of the experiment would also help determine if all treated animals would eventually have increased step counts.
- Further research is needed on other diseases and how best these findings using motion sensor technology could be put to practical use in cattle disease management, prevention or control decisions.

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APPENDICES

Appendix 1a: Principal Component Loadings for Standing Variable in Petauke District (2006/07). Each movement behaviour variable's highest (absolute) loading is bold and underlined.

Variable Standing	*PC1*	*PC2*	*PC3*	*PC4*	*PC5*	*PC6*	*PC7*	*PC8*	*PC9*	*PC10*	*PC11*	*PC12*	*PC13*
0:00	**0.179**	0.131	-0.05	0.004	-0.073	-0.025	-0.036	-0.012	0.063	0.052	0.02	0.053	0.046
1:00	**0.174**	0.11	0.007	-0.02	-0.039	0.026	0.025	0.122	0.055	0.026	0.142	0.134	0.128
2:00	**0.181**	0.09	-0.096	-0.011	0.033	0.047	-0.054	-0.041	0.068	0.01	-0.038	0.046	0.056
3:00	**0.191**	0.063	-0.065	0	0.004	-0.094	-0.076	-0.043	-0.026	0.037	-0.037	0.084	-0.056
4:00	**0.171**	0.135	0.002	0.053	0.016	-0.028	-0.072	-0.129	0	-0.029	-0.028	-0.039	0.033
5:00	0.176	0.096	0.048	0.075	-0.013	-0.039	-0.078	0.006	0.007	-0.083	**0.199**	-0.08	0.079
6:00	0.118	0.07	0.101	0.185	-0.118	-0.056	0.101	0.049	**0.239**	0.096	0.104	-0.177	-0.013
7:00	0.113	-0.035	0.209	-0.039	-0.026	-0.102	-0.102	-0.028	0.134	0.028	-0.185	-0.232	**-0.233**
8:00	0.138	-0.107	0.11	-0.139	0.044	-0.048	-0.122	0.054	0.031	-0.07	**-0.168**	0.031	-0.059
9:00	0.14	-0.118	0.114	-0.136	0.019	-0.094	-0.009	-0.063	0.063	**-0.189**	0.014	0.061	0.161
10:00	0.114	-0.112	0.162	**-0.196**	-0.009	-0.064	0.011	-0.047	-0.022	-0.088	0.032	-0.037	-0.009
11:00	0.118	-0.084	**0.183**	-0.16	-0.059	-0.072	0.109	-0.091	-0.054	-0.004	0.043	0.126	-0.017
12:00	0.125	-0.061	0.161	-0.144	-0.073	-0.041	0.072	-0.09	-0.104	0.142	0.024	**0.167**	-0.024
13:00	0.115	-0.017	0.176	-0.186	0.021	0.042	**0.187**	-0.105	0.054	0.031	0.099	0.009	-0.124
14:00	0.046	0.024	0.187	-0.079	0.18	0.138	**0.251**	-0.007	0.222	0.247	0.045	-0.015	0.023
15:00	-0.02	0.084	0.236	-0.061	0.066	0.235	-0.013	-0.015	0.103	0.039	**-0.241**	-0.039	0.091
16:00	-0.023	0.047	0.23	-0.05	0.033	**0.275**	-0.059	0.044	-0.064	-0.226	0.124	0.102	-0.091
17:00	-0.001	-0.017	0.193	0.138	0.119	**0.259**	0.064	0.028	0.228	-0.079	-0.008	-0.079	-0.062
18:00	0.081	0.071	0.142	0.222	-0.079	0.045	0.193	0.017	**-0.272**	-0.056	-0.175	0.004	0.012
19:00	0.093	-0.01	0.11	0.144	-0.048	0.197	-0.114	-0.05	**-0.313**	-0.048	0.105	-0.307	-0.172
20:00	0.131	0.107	0.024	-0.002	0.169	-0.049	-0.082	0.104	-0.097	0.132	-0.061	0.215	**-0.385**
21:00	**0.173**	0.133	-0.041	-0.033	0.086	-0.084	0.02	0.015	-0.052	0	-0.069	-0.124	0.012
22:00	**0.178**	0.143	-0.01	-0.002	0.005	0.02	-0.097	-0.01	0.026	0.014	0.019	0.035	0.12
23:00	**0.18**	0.105	-0.009	0.028	-0.009	0.018	-0.107	0.091	-0.073	-0.041	0.005	-0.128	0.091

Appendix 1b: Principal Component Loadings for Walking Variable in Petauke District (2006/07). Each movement behaviour variable's highest (absolute) loading is bold and underlined.

Variable Walking	*PC1*	*PC2*	*PC3*	*PC4*	*PC5*	*PC6*	*PC7*	*PC8*	*PC9*	*PC10*	*PC11*	*PC12*	*PC13*
0:00	0.066	-0.015	-0.157	-0.182	-0.142	0.183	-0.007	**0.233**	0.009	-0.073	-0.19	0.078	0.019
1:00	0.073	-0.043	-0.13	-0.223	-0.118	**0.247**	0.031	0.193	-0.006	-0.063	0.001	0.007	0.038
2:00	0.017	-0.049	-0.18	-0.157	-0.136	0.221	-0.082	-0.154	0.17	-0.007	-0.027	**-0.232**	-0.088
3:00	0.006	-0.019	**-0.222**	-0.185	-0.127	0.137	-0.071	-0.145	0.097	0.028	-0.184	-0.185	-0.1
4:00	0.071	-0.039	-0.147	0.006	-0.219	0.018	0.3	-0.104	-0.099	0.09	**-0.264**	-0.096	-0.056
5:00	0.064	-0.018	0.002	0.142	-0.262	-0.018	**0.312**	-0.009	-0.103	0.194	-0.185	0.083	0.003
6:00	-0.036	0.02	-0.008	0.196	**-0.291**	0.092	0.004	0.164	-0.053	-0.074	0.072	0.241	-0.25
7:00	-0.131	0.104	-0.028	0.168	-0.081	0.016	0.038	0.157	0.188	-0.155	0.009	**0.191**	0.139
8:00	-0.111	**0.205**	-0.075	0.139	-0.008	-0.015	0.067	-0.105	0.053	-0.109	-0.03	0.05	0.01
9:00	-0.128	**0.208**	-0.07	0.068	0.054	0.064	0.029	-0.088	-0.063	0.042	-0.056	0.077	-0.116
10:00	-0.131	**0.194**	-0.101	0.058	0.044	0.115	-0.041	-0.054	0.003	0.107	-0.006	0.089	-0.062
11:00	-0.132	**0.208**	-0.096	0.05	0.037	0.077	-0.062	-0.026	0.024	0.105	-0.017	-0.079	-0.041
12:00	-0.13	**0.196**	-0.073	0.077	0.052	0.089	-0.064	0.044	-0.007	0.029	0.016	-0.158	0.04
13:00	-0.132	0.14	-0.011	0.014	0.073	0.01	-0.01	0.265	-0.145	0.067	-0.091	-0.082	**0.315**
14:00	-0.108	0.042	0.087	-0.044	-0.118	-0.231	-0.214	0.142	-0.143	-0.051	**-0.307**	-0.069	0.052
15:00	-0.093	0.063	0.072	-0.074	**-0.263**	-0.14	-0.132	-0.135	-0.143	-0.261	0.16	-0.028	0.052
16:00	-0.081	0.11	-0.102	-0.077	**-0.264**	-0.109	0.09	-0.193	0.082	0.07	-0.001	-0.113	0.123
17:00	-0.06	0.107	-0.019	-0.216	**-0.26**	-0.164	0.054	-0.004	-0.153	0.04	0.014	0.092	0.009
18:00	-0.104	0.034	-0.066	-0.216	-0.069	-0.146	-0.179	0.086	**0.261**	0.127	0.186	-0.062	-0.129
19:00	-0.104	0.094	-0.097	-0.2	0.068	**-0.21**	0.078	0.157	0.183	0.005	0.036	0.158	0.048
20:00	-0.057	0.083	-0.09	-0.065	0.127	-0.096	**0.329**	0.063	0.002	-0.309	0.003	-0.312	-0.004
21:00	0.049	0.064	-0.162	-0.078	0.042	-0.07	**0.385**	-0.043	0.064	-0.27	0.097	-0.038	-0.057
22:00	0.083	0.04	-0.156	-0.133	-0.055	**0.251**	0.004	0.081	0.068	-0.024	-0.085	-0.018	0.005
23:00	0.09	-0.042	-0.081	-0.101	-0.099	0.204	0.004	**0.327**	-0.081	-0.112	-0.101	0.107	-0.145

Appendix 1c: Principal Component Loadings for Lying Variable in Petauke District (2006/07). Each movement behaviour variable's highest (absolute) loading is bold and underlined.

Variable Lying	*PC1*	*PC2*	*PC3*	*PC4*	*PC5*	*PC6*	*PC7*	*PC8*	*PC9*	*PC10*	*PC11*	*PC12*	*PC13*
0:00	**-0.18**	-0.128	0.057	0.004	0.079	0.016	0.036	0.001	-0.063	-0.048	-0.011	-0.056	-0.046
1:00	**-0.174**	-0.106	0	0.03	0.044	-0.037	-0.026	-0.129	-0.053	-0.023	-0.139	-0.132	-0.127
2:00	**-0.178**	-0.083	0.11	0.025	-0.02	-0.066	0.06	0.054	-0.081	-0.009	0.04	-0.023	-0.047
3:00	**-0.189**	-0.061	0.082	0.015	0.007	0.082	0.081	0.054	0.018	-0.039	0.052	-0.068	0.063
4:00	**-0.173**	-0.13	0.008	-0.053	-0.001	0.026	0.051	0.134	0.006	0.022	0.046	0.045	-0.029
5:00	**-0.179**	-0.08	-0.044	-0.114	0.098	0.041	-0.034	-0.002	0.028	0.01	-0.117	0.044	-0.071
6:00	-0.091	-0.069	-0.086	**-0.235**	0.211	0.016	-0.09	-0.104	-0.191	-0.058	-0.118	0.067	0.104
7:00	0.029	-0.078	-0.184	-0.145	0.116	0.088	0.063	-0.145	**-0.345**	0.143	0.18	0.028	0.087
8:00	-0.005	-0.207	-0.028	-0.042	-0.051	0.097	0.059	0.107	-0.14	0.296	**0.299**	-0.134	0.069
9:00	0.04	-0.216	-0.028	0.063	-0.123	0.013	-0.041	**0.244**	0.028	0.182	0.083	-0.223	-0.008
10:00	0.09	**-0.2**	-0.023	0.138	-0.066	-0.122	0.059	0.152	0.022	-0.078	-0.029	-0.11	0.12
11:00	0.072	**-0.233**	-0.061	0.107	0.01	-0.038	-0.029	0.148	0.025	-0.162	-0.024	-0.022	0.085
12:00	0.049	**-0.24**	-0.09	0.061	0.009	-0.091	0.01	0.044	0.146	-0.23	-0.057	0.04	-0.034
13:00	0.036	-0.142	-0.163	0.17	-0.104	-0.054	-0.176	-0.197	0.111	-0.107	0.004	0.084	**-0.236**
14:00	0.07	-0.062	-0.242	0.109	-0.03	0.118	0.008	-0.137	-0.039	-0.152	**0.272**	0.081	-0.072
15:00	0.08	-0.107	**-0.226**	0.098	0.136	-0.076	0.103	0.107	0.024	0.155	0.066	0.048	-0.104
16:00	0.087	-0.133	-0.147	0.11	0.176	**-0.186**	-0.013	0.109	-0.002	0.168	-0.122	-0.012	-0.007
17:00	0.078	-0.118	-0.186	0.125	**0.202**	-0.073	-0.139	-0.025	-0.053	0.036	-0.01	-0.031	0.056
18:00	0.06	-0.151	-0.086	0.053	**0.221**	0.177	0.031	-0.163	-0.056	-0.132	-0.067	0.096	0.195
19:00	0.054	-0.169	0.011	0.159	-0.056	0.091	0.033	-0.227	0.15	0.069	**-0.242**	0.19	0.185
20:00	-0.073	-0.152	0.044	0.048	-0.238	0.112	-0.165	-0.135	0.083	0.106	0.051	0.037	**0.337**
21:00	**-0.168**	-0.134	0.069	0.045	-0.087	0.091	-0.093	-0.005	0.035	0.053	0.044	0.12	0
22:00	**-0.178**	-0.141	0.019	0.01	-0.002	-0.035	0.094	0.005	-0.03	-0.012	-0.013	-0.033	-0.117
23:00	**-0.181**	-0.101	0.012	-0.023	0.013	-0.027	0.105	-0.104	0.075	0.046	0	0.12	-0.083

Appendix 2a: Principal Component Loadings for Standing Variables in Treatment Study. Each movement behaviour variable's highest (absolute) loading is bold and underlined.

Variable Standing	*PC1*	*PC2*	*PC3*	*PC4*	*PC5*	*PC6*	*PC7*	*PC8*	*PC9*	*PC10*	*PC11*	*PC12*
0:00	**0.189**	0.035	-0.007	0.048	-0.101	-0.030	0.006	-0.058	-0.144	-0.021	0.064	0.011
1:00	**0.189**	0.044	-0.067	-0.006	-0.027	-0.071	0.028	-0.029	-0.066	0.074	0.013	-0.019
2:00	**0.180**	0.110	-0.082	0.011	-0.046	0.008	0.035	0.038	-0.088	-0.078	0.018	-0.066
3:00	**0.177**	0.086	-0.079	0.004	-0.119	-0.042	0.076	0.005	-0.064	-0.022	0.070	0.053
4:00	0.175	0.050	-0.089	0.015	-0.024	-0.092	-0.086	0.002	-0.138	0.043	-0.027	**-0.194**
5:00	**0.175**	0.004	-0.086	-0.048	0.011	-0.052	-0.051	0.152	0.023	-0.063	0.100	-0.154
6:00	0.081	-0.018	-0.150	-0.208	0.035	-0.121	0.152	**0.238**	0.135	-0.133	0.007	-0.195
7:00	0.032	0.156	-0.151	-0.075	**0.223**	-0.152	0.113	0.062	0.185	-0.029	0.017	-0.108
8:00	0.001	0.224	-0.013	0.010	**0.230**	0.115	0.161	-0.096	-0.020	-0.071	0.050	0.074
9:00	-0.040	**0.211**	-0.064	0.055	0.196	0.198	0.056	-0.102	-0.052	0.010	0.119	0.140
10:00	-0.070	0.177	-0.044	-0.039	0.019	0.187	0.157	**-0.199**	0.063	0.110	0.165	-0.069
11:00	-0.067	0.101	-0.021	**-0.291**	-0.024	0.121	0.125	0.107	-0.029	0.211	0.006	-0.074
12:00	-0.091	0.150	-0.001	-0.202	-0.143	-0.025	0.024	-0.076	0.089	0.208	0.128	**-0.233**
13:00	-0.058	0.128	-0.054	**-0.248**	-0.115	0.001	0.035	-0.157	0.040	0.164	-0.123	0.075
14:00	-0.115	0.087	-0.121	-0.152	-0.162	-0.096	**-0.180**	-0.012	-0.021	0.087	0.001	0.179
15:00	-0.078	0.106	-0.205	-0.052	-0.069	0.073	**-0.278**	0.020	0.071	-0.143	0.124	0.054
16:00	-0.100	0.111	-0.182	0.144	-0.033	0.125	-0.026	0.113	-0.081	-0.017	**0.191**	-0.062
17:00	-0.037	0.153	-0.212	0.105	0.047	0.118	-0.002	0.211	0.029	-0.061	**-0.239**	-0.023
18:00	0.143	0.077	0.092	-0.012	0.076	0.154	0.058	-0.093	0.096	-0.152	**-0.252**	-0.052
19:00	**0.182**	0.080	0.025	-0.063	-0.048	0.070	0.053	0.023	0.082	0.010	0.050	0.149
20:00	**0.177**	0.079	-0.043	0.015	-0.092	0.031	-0.067	-0.127	0.027	-0.047	-0.058	0.087
21:00	**0.183**	0.050	-0.017	-0.020	-0.114	0.017	-0.040	0.007	0.145	0.066	-0.123	0.145
22:00	**0.189**	0.053	0.031	0.028	-0.076	0.017	-0.028	-0.109	0.019	0.020	-0.068	-0.076
23:00	**0.190**	0.054	-0.025	0.023	-0.087	0.016	0.048	-0.104	-0.051	0.036	0.104	0.069

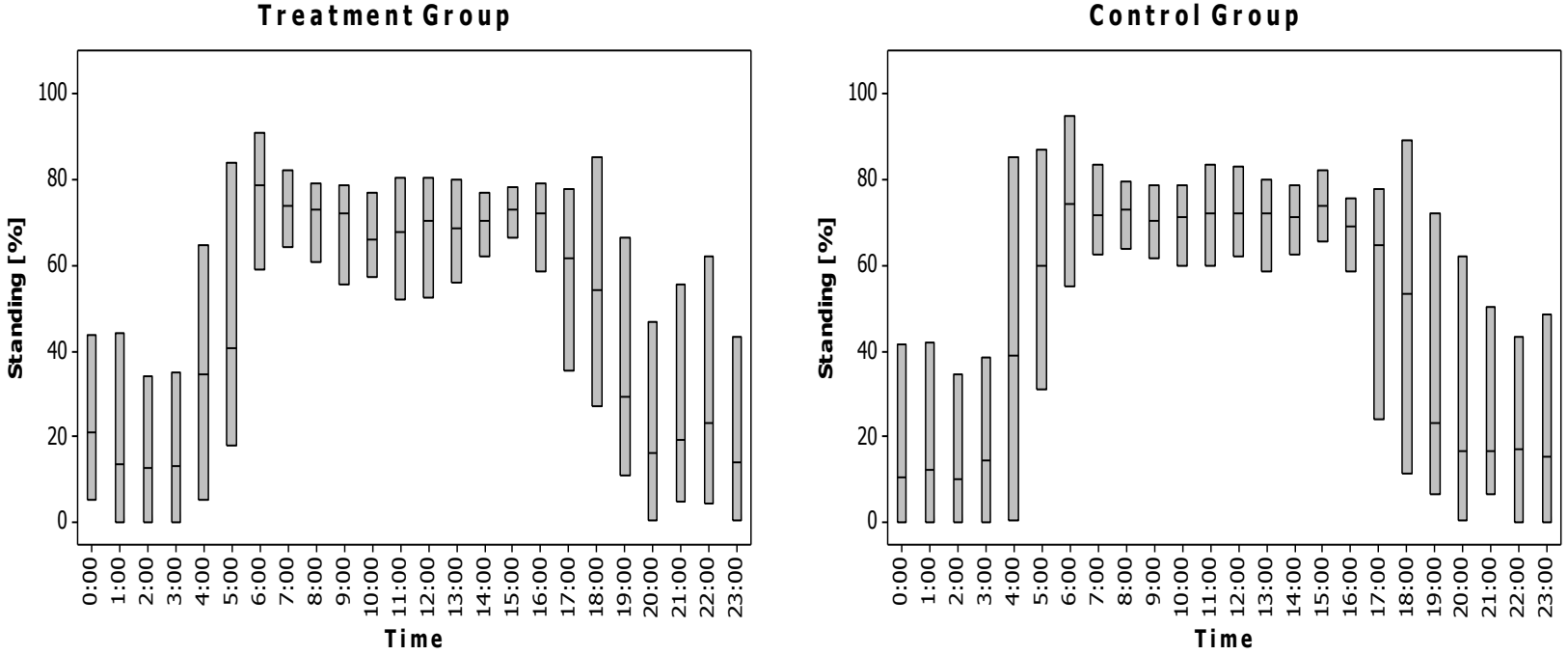
Appendix 2b: Principal Component Loadings for Walking Variables in Treatment Study. Each movement behaviour variable's highest (absolute) loading is bold and underlined.

Variable Walking	*PC1*	*PC2*	*PC3*	*PC4*	*PC5*	*PC6*	*PC7*	*PC8*	*PC9*	*PC10*	*PC11*	*PC12*
0:00	0.049	**-0.207**	-0.135	-0.012	0.080	0.113	-0.057	-0.126	-0.127	0.052	0.106	0.034
1:00	-0.006	-0.183	**-0.245**	-0.073	0.062	0.050	0.013	-0.053	-0.172	0.062	-0.058	-0.032
2:00	0.018	-0.168	**-0.225**	-0.079	0.117	0.150	0.047	0.029	-0.214	0.107	-0.001	-0.013
3:00	-0.004	-0.116	-0.252	-0.051	-0.053	0.078	0.057	0.193	-0.152	-0.095	0.017	**0.347**
4:00	-0.002	-0.161	-0.188	-0.064	0.050	0.037	-0.026	-0.140	-0.233	0.167	-0.218	**-0.234**
5:00	0.054	**-0.238**	-0.045	-0.098	-0.056	0.059	-0.165	0.045	0.129	-0.087	0.182	0.012
6:00	0.051	-0.231	0.078	-0.002	-0.084	0.059	0.113	-0.146	0.023	-0.021	**0.246**	0.127
7:00	0.010	-0.209	0.093	0.058	-0.097	-0.100	**0.258**	-0.061	-0.107	0.108	0.116	0.134
8:00	-0.052	**-0.146**	-0.113	0.091	-0.111	-0.301	0.085	0.037	-0.033	0.092	-0.121	0.099
9:00	-0.053	-0.145	-0.128	-0.016	-0.166	**-0.284**	0.048	-0.087	0.143	-0.036	-0.222	-0.035
10:00	-0.086	-0.107	-0.141	-0.065	-0.203	-0.165	0.002	-0.060	0.095	**-0.337**	-0.097	0.028
11:00	-0.122	-0.019	-0.021	-0.042	-0.150	-0.053	-0.012	-0.280	-0.035	**-0.357**	0.110	-0.061
12:00	0.005	-0.104	0.026	-0.222	0.041	0.152	0.093	0.024	-0.277	**-0.302**	-0.197	0.123
13:00	0.079	-0.061	0.175	**-0.195**	0.095	0.047	-0.032	0.186	-0.187	-0.109	-0.147	0.111
14:00	0.096	-0.005	**0.217**	-0.155	0.184	0.001	0.041	0.138	-0.015	0.040	0.070	0.041
15:00	0.101	-0.013	0.129	-0.099	0.217	**-0.247**	-0.094	-0.036	-0.068	0.183	0.069	0.003
16:00	0.098	-0.043	0.098	-0.156	0.197	**-0.240**	-0.007	-0.179	0.014	-0.045	-0.152	0.117
17:00	0.043	-0.118	0.180	-0.128	0.098	-0.164	-0.108	**-0.273**	-0.041	-0.033	0.214	0.019
18:00	0.065	-0.132	0.152	-0.113	0.048	0.092	0.095	-0.047	0.024	**-0.238**	0.157	-0.066
19:00	0.084	-0.143	-0.112	-0.087	0.165	0.123	0.029	0.144	**0.265**	0.014	0.129	0.157
20:00	0.077	-0.168	-0.153	-0.015	0.078	0.147	-0.101	-0.171	**0.225**	0.068	-0.024	0.058
21:00	0.087	-0.151	-0.095	-0.042	0.061	0.105	-0.062	-0.040	**0.369**	0.167	-0.127	0.084
22:00	0.051	-0.200	-0.083	-0.035	0.163	0.134	-0.051	-0.217	0.081	0.119	-0.084	**-0.222**
23:00	0.023	-0.201	**-0.220**	-0.010	0.057	0.143	0.043	-0.064	-0.079	0.085	0.053	0.054

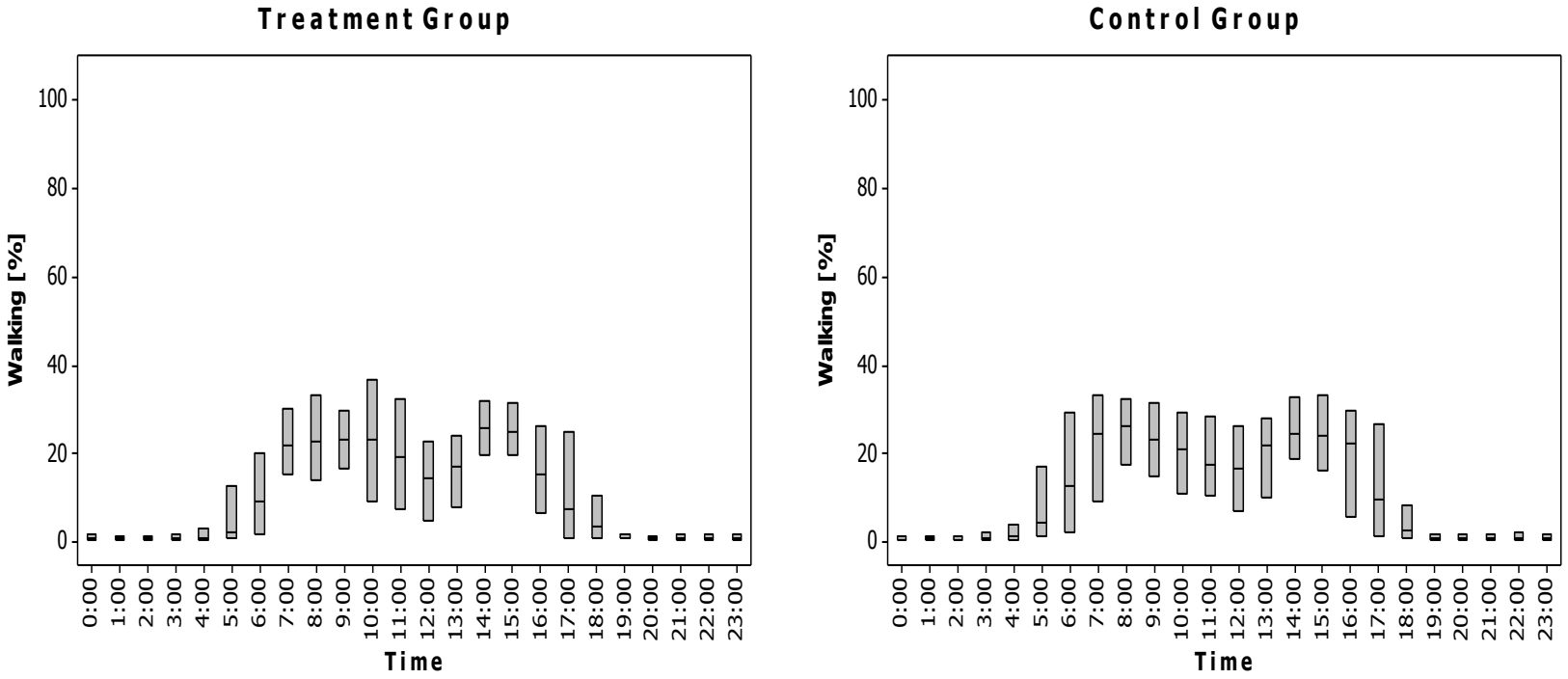
Appendix 2c: Principal Component Loadings for Lying Variables in Treatment Study. Each movement behaviour variable's highest (absolute) loading is bold and underlined.

Variable Lying	*PC1*	*PC2*	*PC3*	*PC4*	*PC5*	*PC6*	*PC7*	*PC8*	*PC9*	*PC10*	*PC11*	*PC12*
0:00	**-0.189**	-0.028	0.012	-0.047	0.097	0.024	-0.003	0.063	0.148	0.023	-0.067	-0.013
1:00	**-0.188**	-0.033	0.082	0.010	0.023	0.068	-0.029	0.032	0.075	-0.079	-0.010	0.021
2:00	**-0.181**	-0.103	0.090	-0.008	0.041	-0.011	-0.038	-0.041	0.095	0.067	-0.019	0.068
3:00	**-0.176**	-0.080	0.091	-0.001	0.121	0.038	-0.078	-0.014	0.071	0.026	-0.070	-0.069
4:00	-0.173	-0.041	0.098	-0.012	0.021	0.088	0.087	0.006	0.150	-0.049	0.039	**0.204**
5:00	**-0.171**	0.029	0.088	0.059	-0.002	0.036	0.073	-0.146	-0.037	0.087	-0.117	0.139
6:00	-0.097	0.118	0.105	**0.194**	0.004	0.086	-0.191	-0.156	-0.136	0.132	-0.114	0.125
7:00	-0.046	0.003	0.092	0.036	-0.171	0.260	**-0.352**	-0.017	-0.118	-0.061	-0.120	0.007
8:00	0.054	-0.114	0.135	-0.108	-0.158	0.179	**-0.282**	0.076	0.059	-0.012	0.068	-0.193
9:00	0.098	-0.110	0.199	-0.050	-0.071	0.040	-0.112	**0.204**	-0.076	0.023	0.074	-0.132
10:00	0.123	-0.076	0.140	0.081	0.130	-0.042	-0.138	**0.216**	-0.123	0.148	-0.073	0.040
11:00	0.125	-0.070	0.029	**0.258**	0.107	-0.066	-0.094	0.077	0.044	0.038	-0.069	0.095
12:00	0.079	-0.071	-0.016	**0.323**	0.104	-0.074	-0.081	0.054	0.095	0.002	0.008	0.133
13:00	0.007	-0.081	-0.055	**0.343**	0.049	-0.029	-0.013	0.034	0.074	-0.086	0.200	-0.135
14:00	0.059	-0.093	-0.018	**0.277**	0.049	0.105	0.170	-0.084	0.034	-0.125	-0.051	-0.227
15:00	0.005	-0.107	0.123	0.137	-0.097	0.117	**0.380**	0.007	-0.023	0.011	-0.191	-0.062
16:00	0.013	-0.125	0.160	0.007	**-0.276**	0.184	0.058	0.103	0.121	0.105	-0.085	-0.088
17:00	-0.011	-0.086	0.083	0.042	**-0.319**	0.093	0.242	0.122	0.024	0.207	0.069	0.012
18:00	-0.145	-0.031	-0.123	0.042	-0.081	-0.158	-0.078	0.093	-0.093	**0.191**	0.177	0.068
19:00	**-0.183**	-0.073	-0.021	0.065	0.042	-0.072	-0.053	-0.029	-0.091	-0.013	-0.054	-0.152
20:00	**-0.178**	-0.072	0.048	-0.014	0.088	-0.036	0.070	0.132	-0.035	0.046	0.059	-0.089
21:00	**-0.183**	-0.044	0.020	0.021	0.109	-0.021	0.042	-0.005	-0.156	-0.070	0.126	-0.147
22:00	**-0.190**	-0.043	-0.027	-0.027	0.069	-0.023	0.030	0.119	-0.023	-0.024	0.071	0.085
23:00	**-0.190**	-0.042	0.038	-0.022	0.083	-0.024	-0.050	0.107	0.056	-0.041	-0.106	-0.071

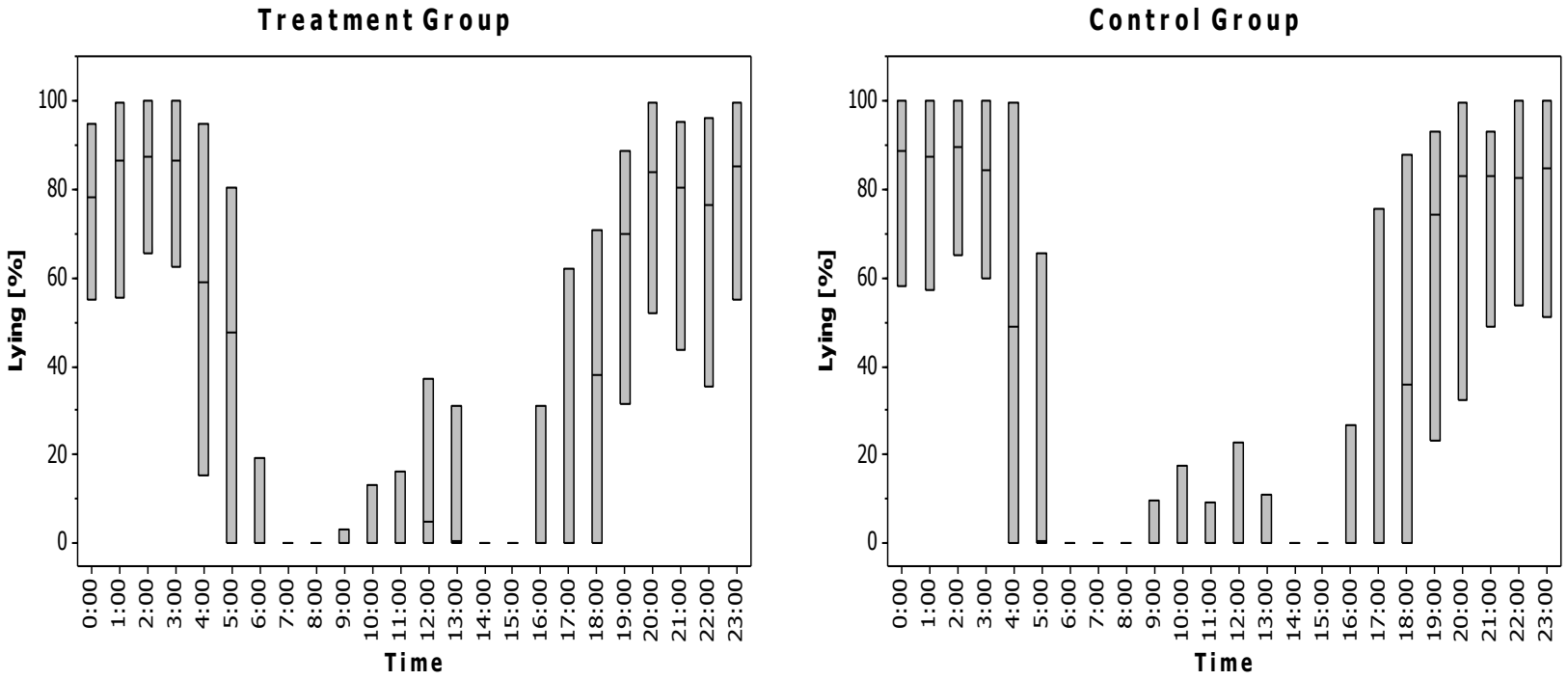
Appendix 3a: Pre-treatment standing behaviour profiles as a mean percentage of time cattle spent standing in each hour of the day in Makale during the one week period before treatment. Each box indicates the lower and upper quartiles representing the points below which 25 % and 75 % of the observations lie respectively. The median value is shown within each box as a horizontal line. This is the value below which half, the observations lie.



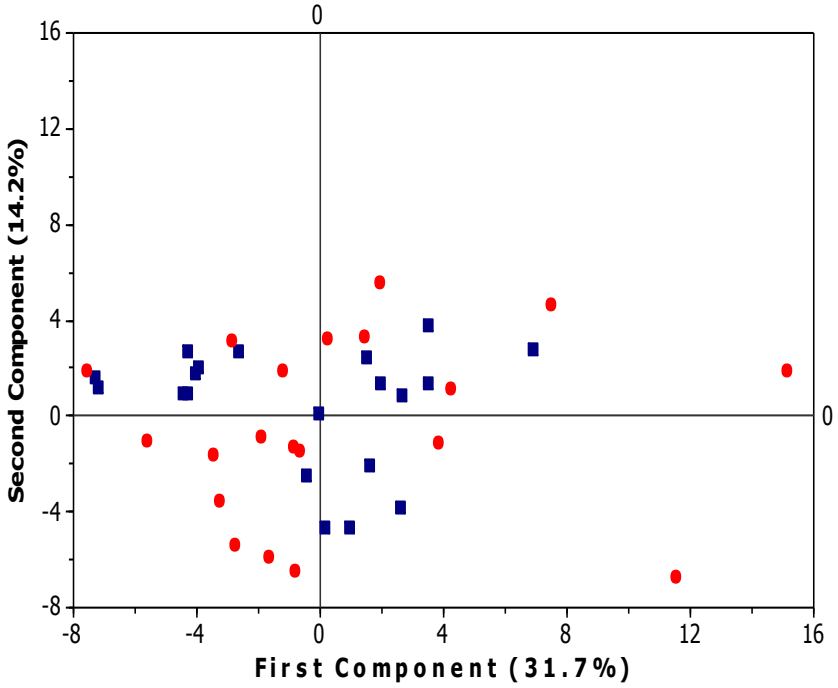
Appendix 3b: Pre-treatment walking behaviour profiles as a mean percentage of time cattle spent walking in each hour of the day in Makale during the one week period before treatment.

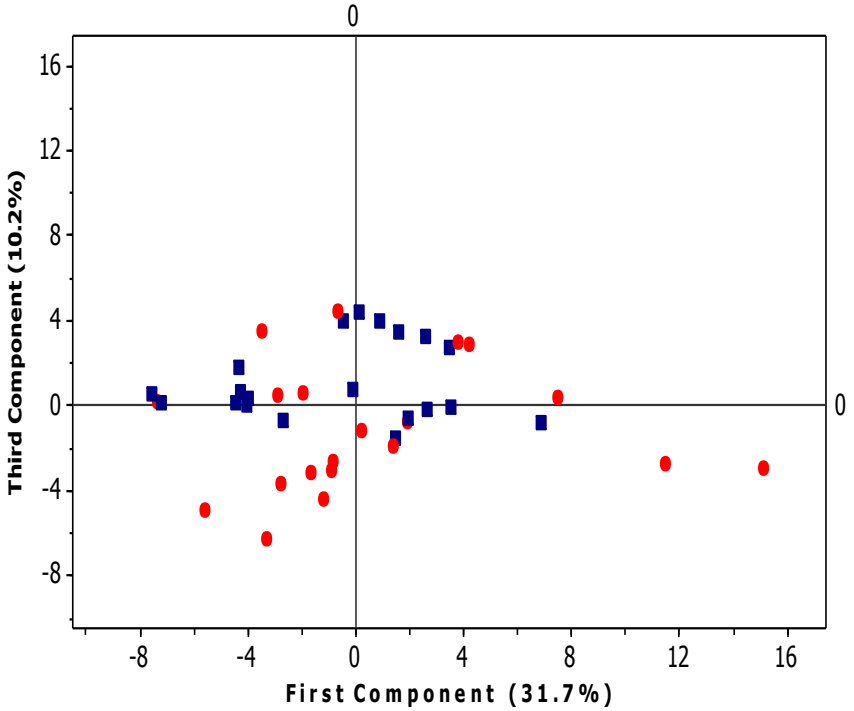


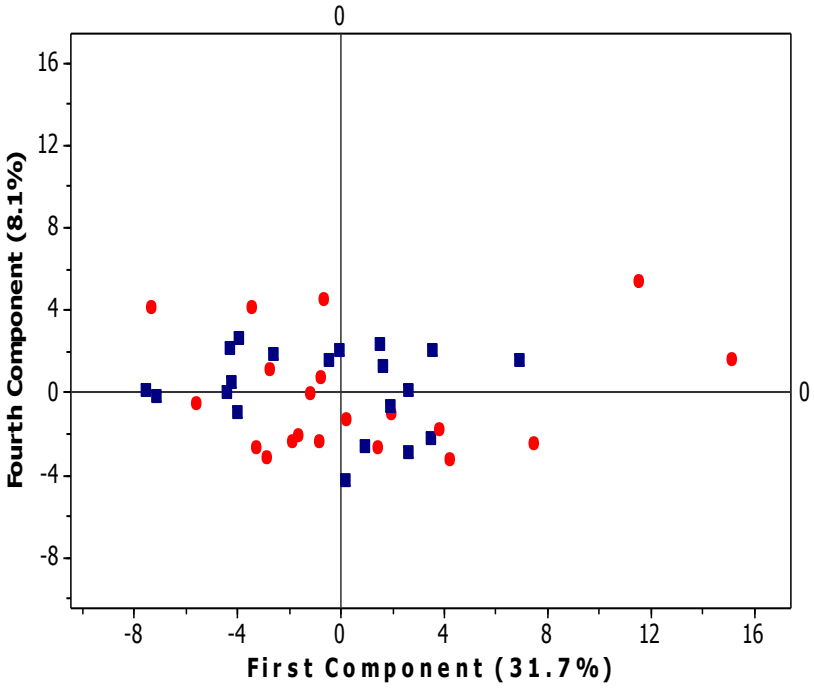
Appendix 3c: Pre-treatment lying behaviour profiles as a mean percentage of time cattle spent lying in each hour of the day in Makale during the one week period before treatment.

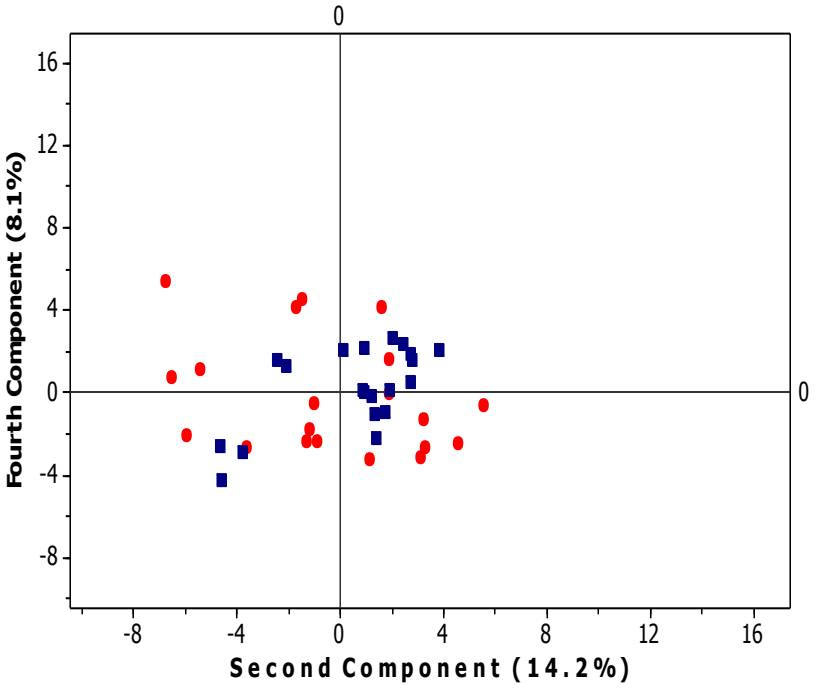


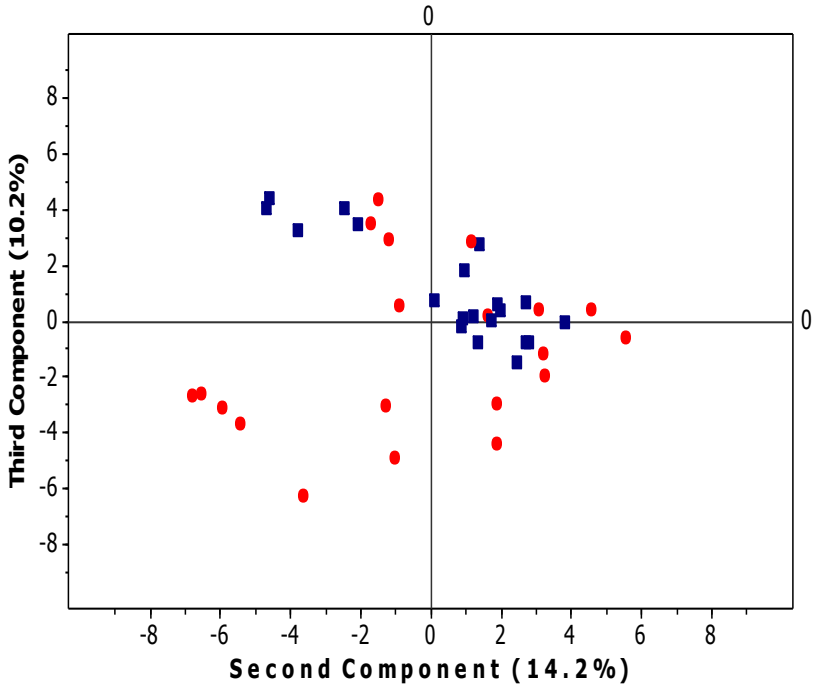
Appendix 4: Score Plots of various combinations of Principal components resulting from the PCA of motion sensor data after treatment of cattle in the treatment group (■) and control group (●) over a period of two weeks in Makale.

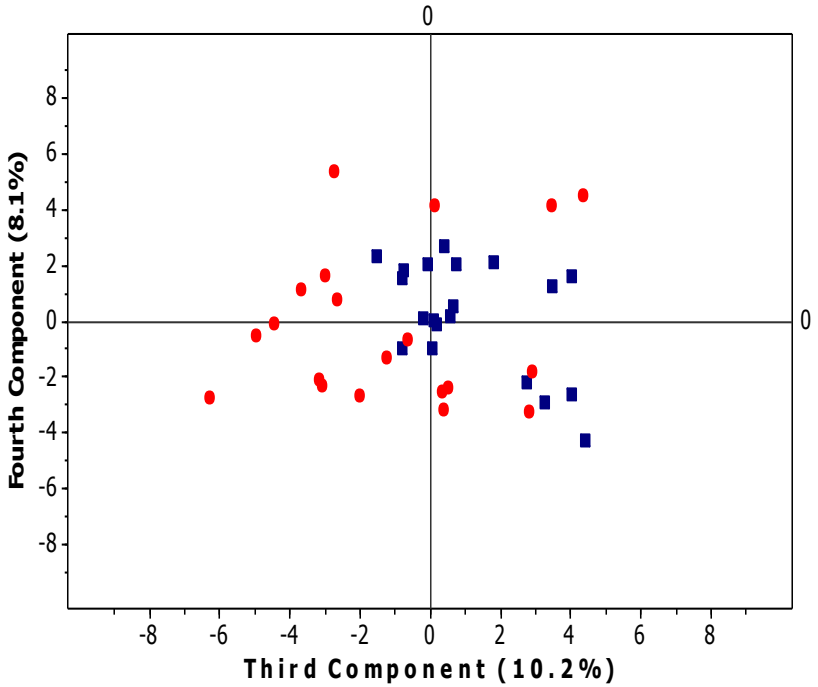




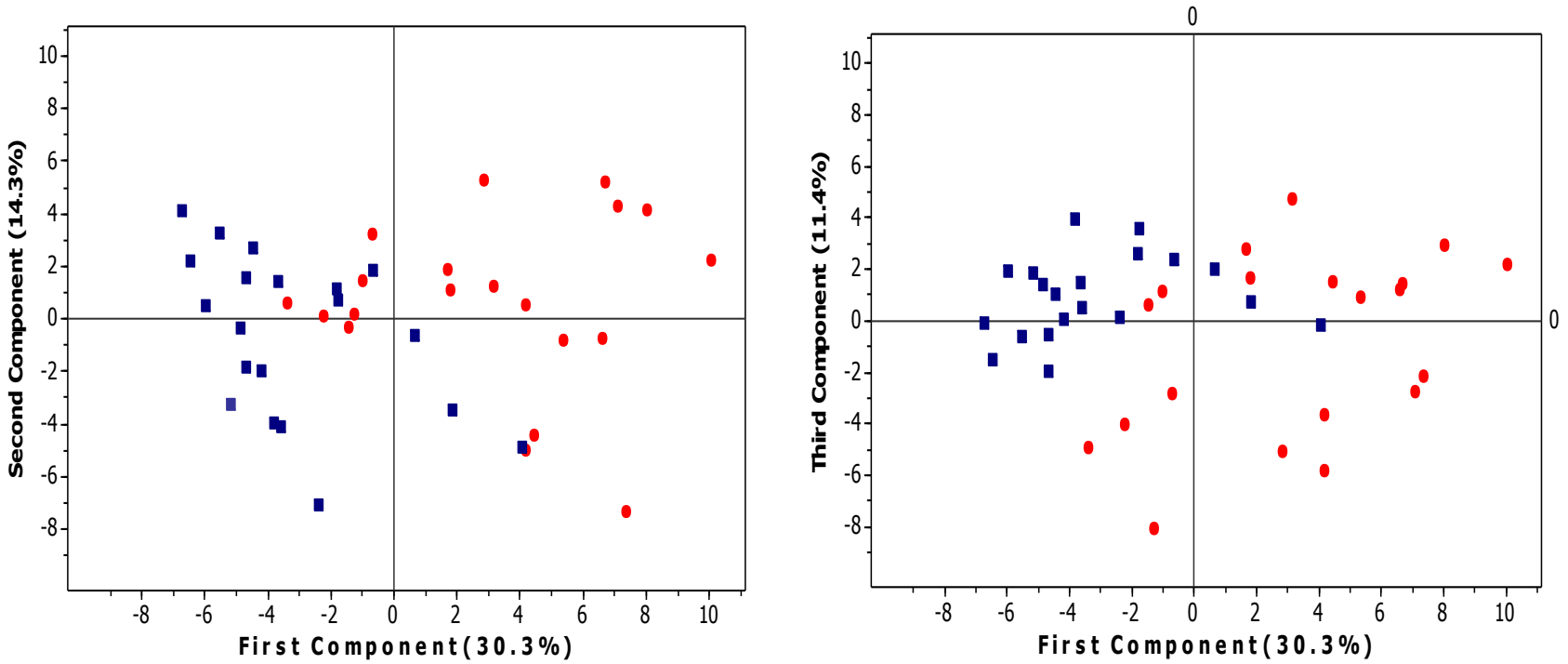

0
16
12
8
4
0
-4
-8
Fourth Component (8.1%)
-8
-4
0
4
8
12
16
First Component (31.7%)
0

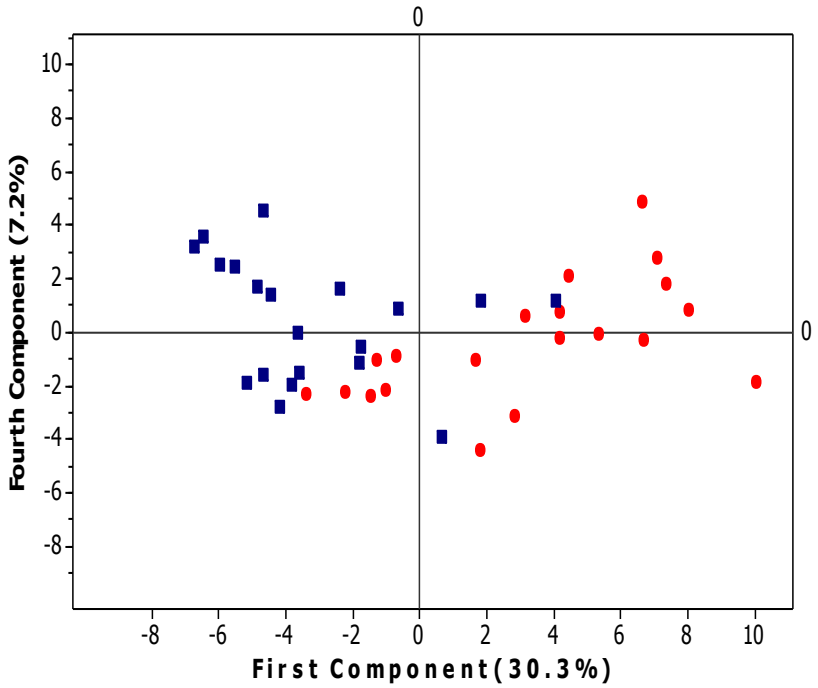

0
16
12
8
4
0
-4
-8
Fourth Component (8.1%)
-8
-4
0
4
8
12
16
Second Component (14.2%)
0

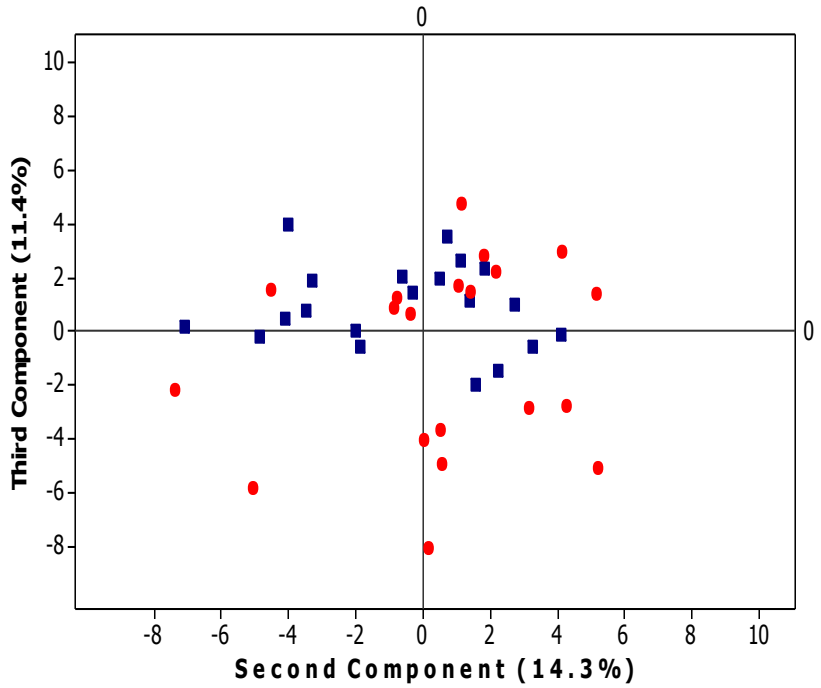

Third Component (10.2%)
Second Component (14.2%)
0
0
8
6
4
2
0
-2
-4
-6
-8
-8
-6
-4
-2
0
2
4
6
8

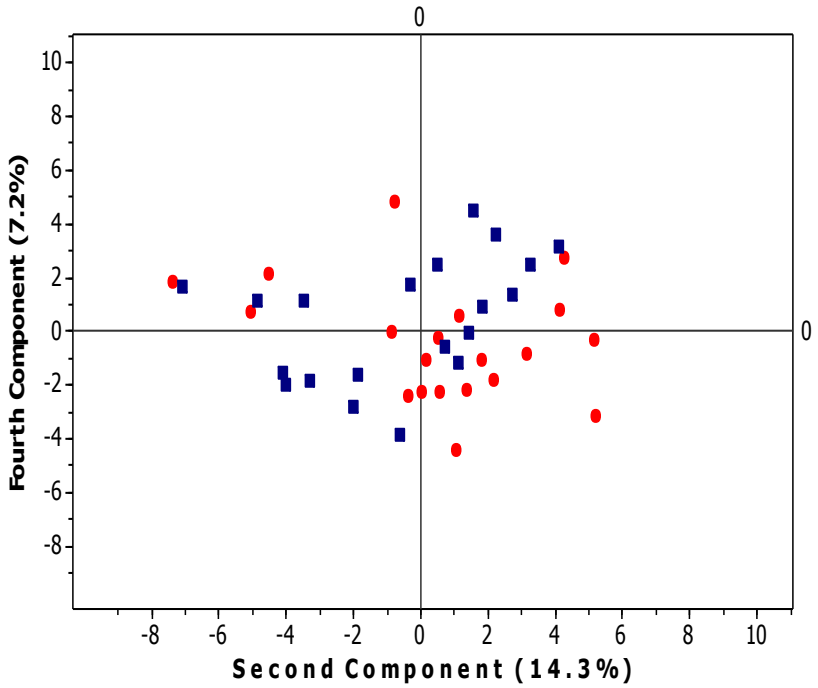

Fourth Component (8.1%)
Third Component (10.2%)
0
0
8
6
4
2
0
-2
-4
-6
-8
-8
-6
-4
-2
0
2
4
6
8

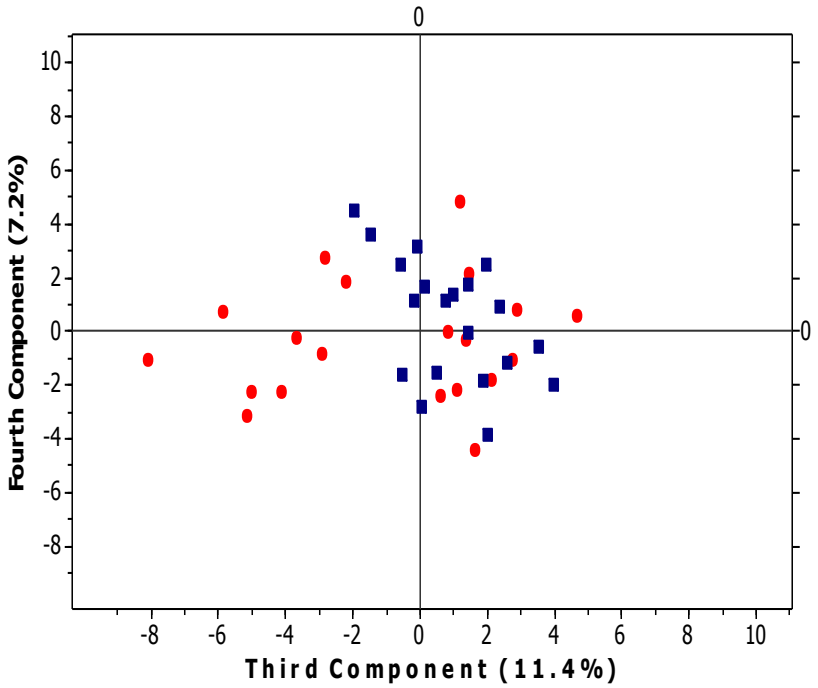
Appendix 5: Score Plots of various combinations of Principal Components resulting from the PCA of motion sensor data of cattle in Kasero (■) and Makale (●) veterinary camps (2006/07 study).



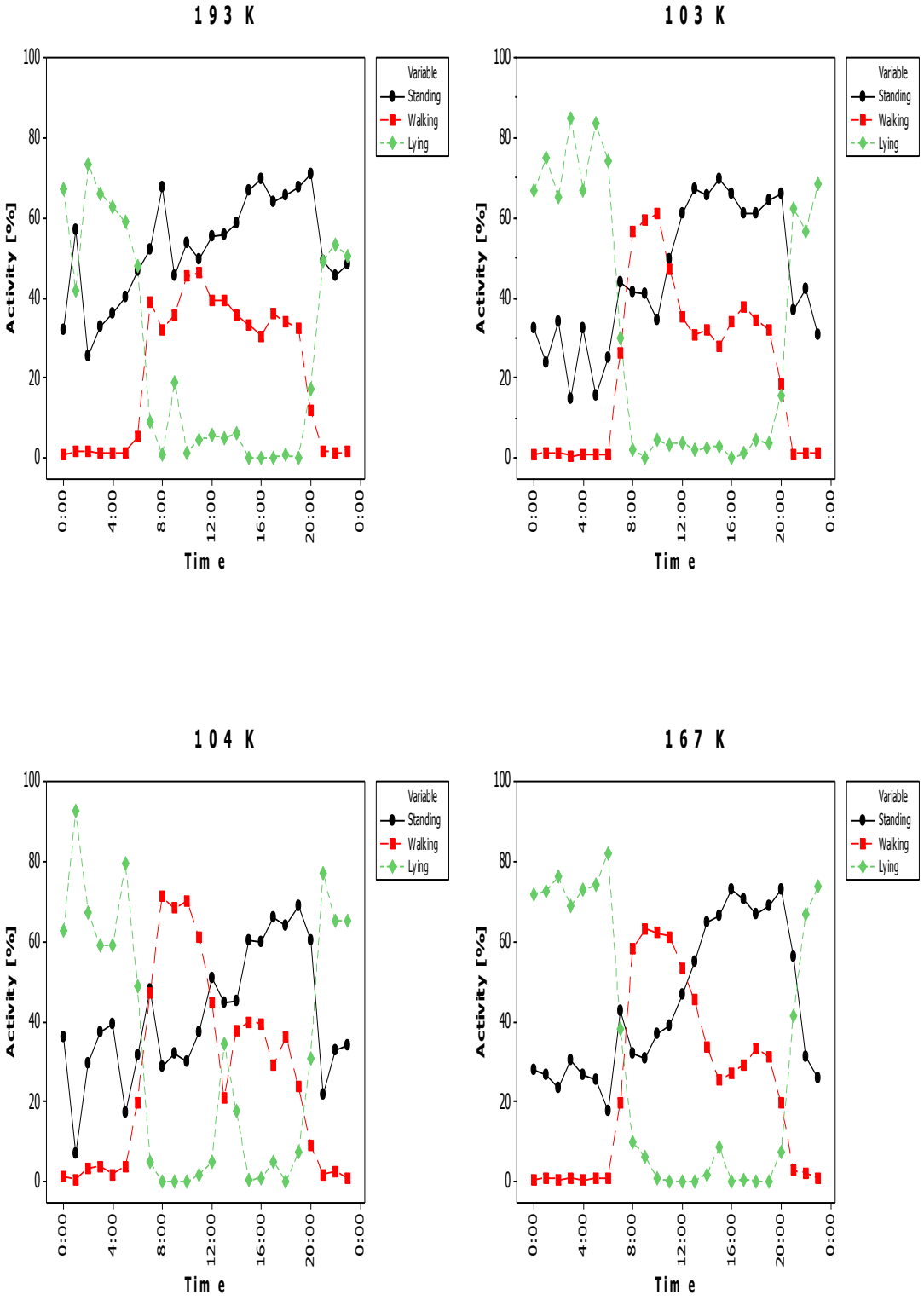

0
10
8
6
4
2
0
-2
-4
-6
-8
0
-8 -6 -4 -2 0 2 4 6 8 10
Fourth Component (7.2%)
First Component (30.3%)

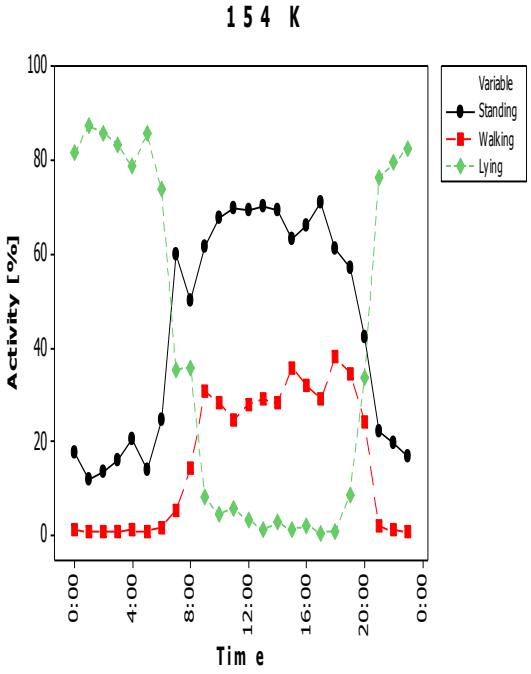

0
10
8
6
4
2
0
-2
-4
-6
-8
0
-8 -6 -4 -2 0 2 4 6 8 10
Third Component (11.4%)
Second Component (14.3%)

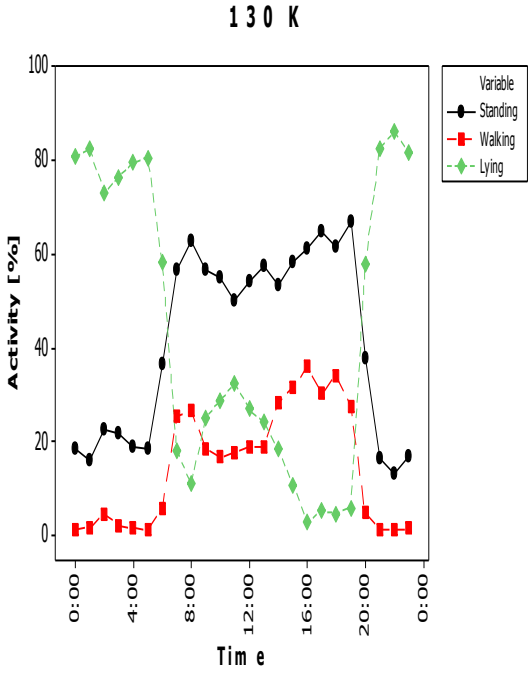

Fourth Component (7.2%)
Second Component (14.3%)

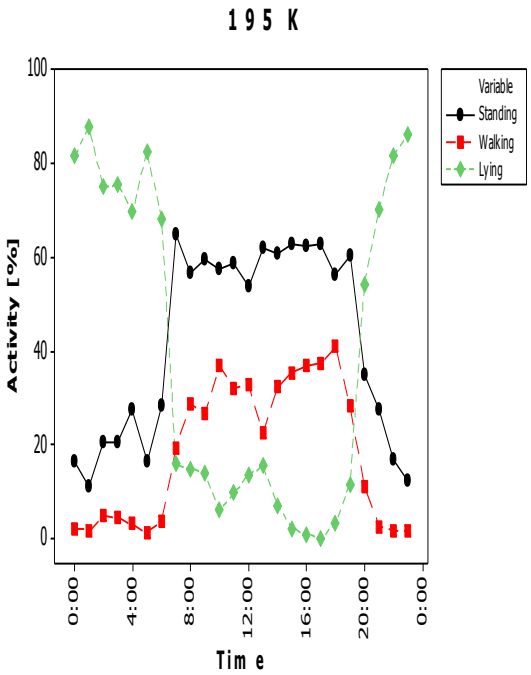

Fourth Component (7.2%)
Third Component (11.4%)

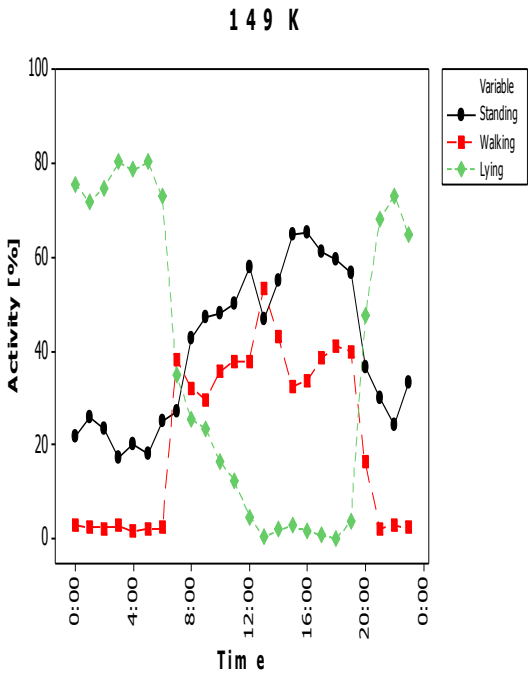
Appendix 6: Movement behaviour profiles of cattle pairs indicating percentage of time spent standing, walking or lying down in Kasero during the two week period during the 2006/07 study. The data point for each behaviour variable at each hour is the mean behaviour of the animal during this period. Cattle on the left are in the low haemoglobin group.

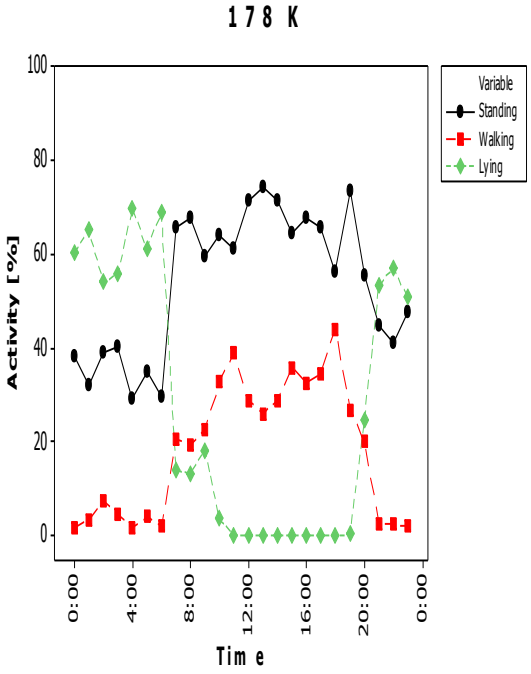


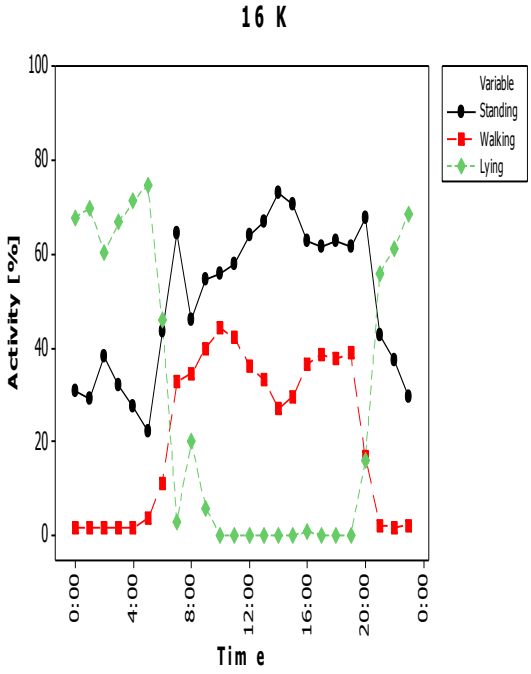

154 K
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00

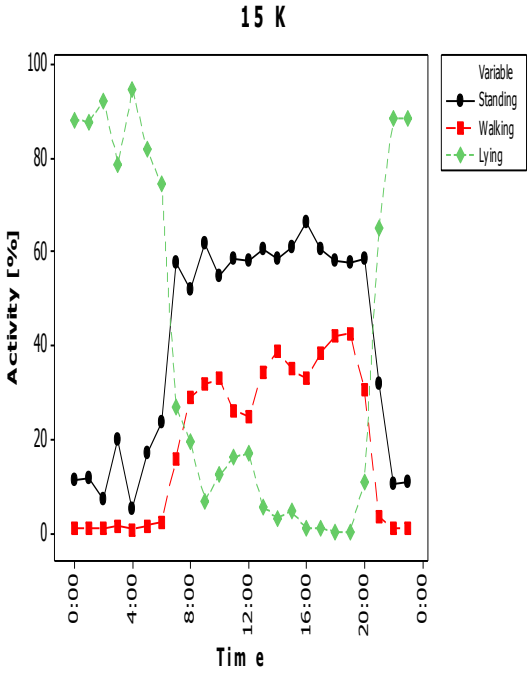

130 K
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00

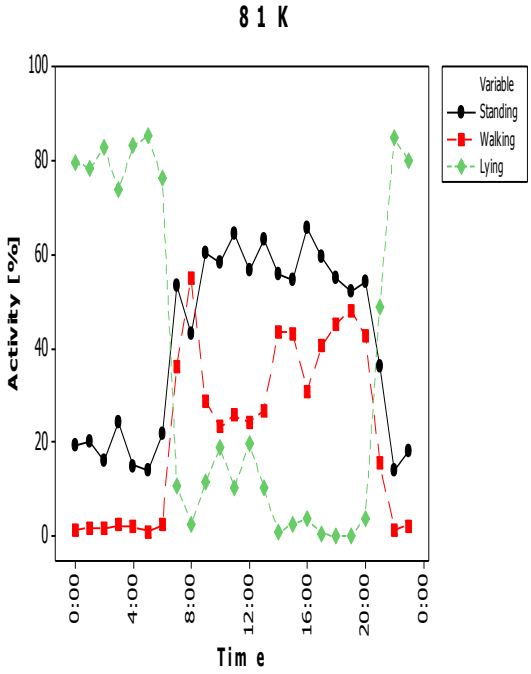

195 K
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00

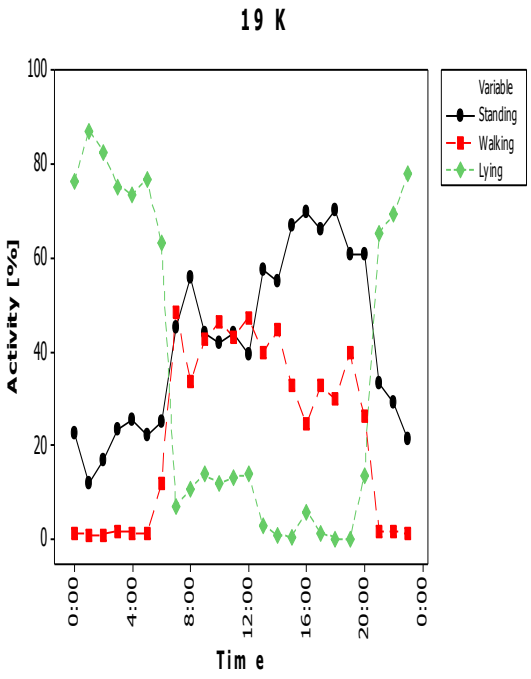

149 K
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00

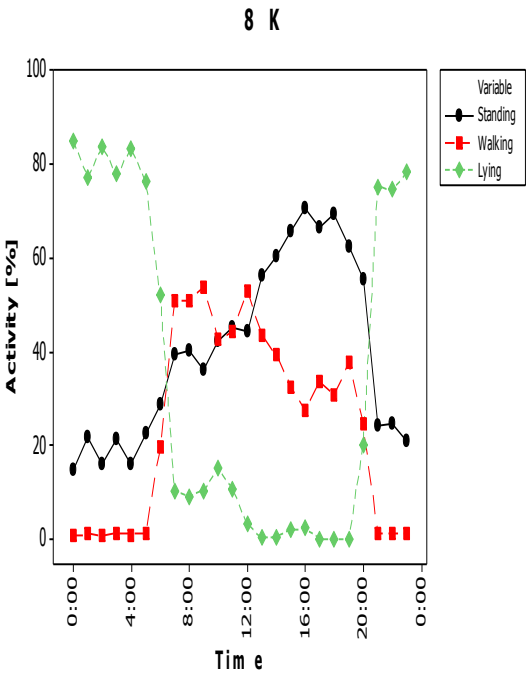

178 K
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00

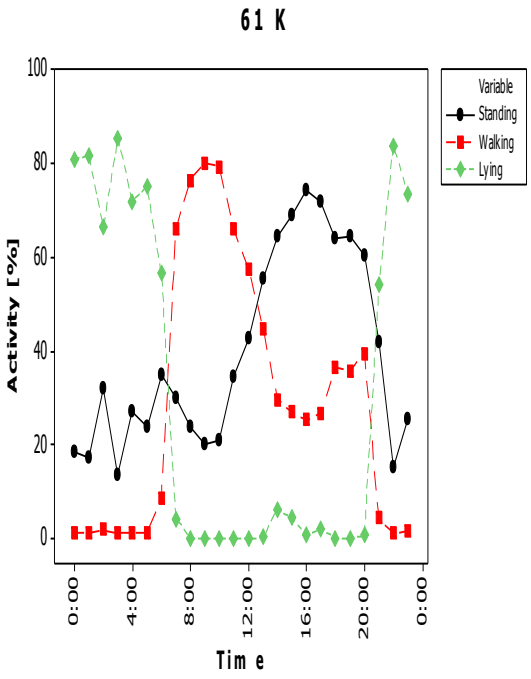

16 K
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00

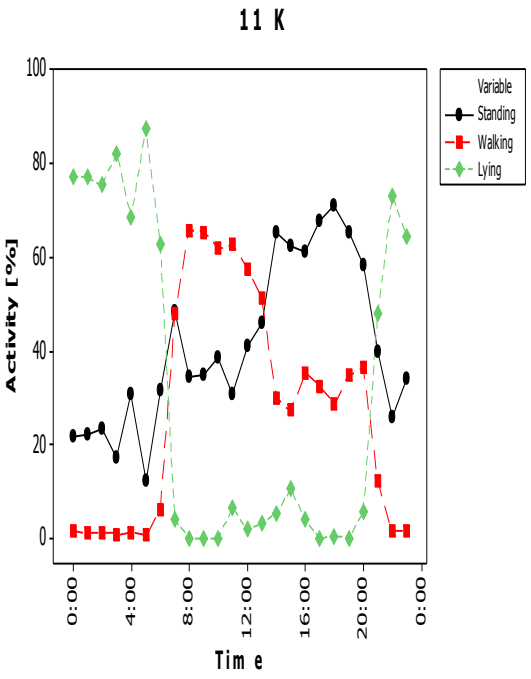

15 K
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00

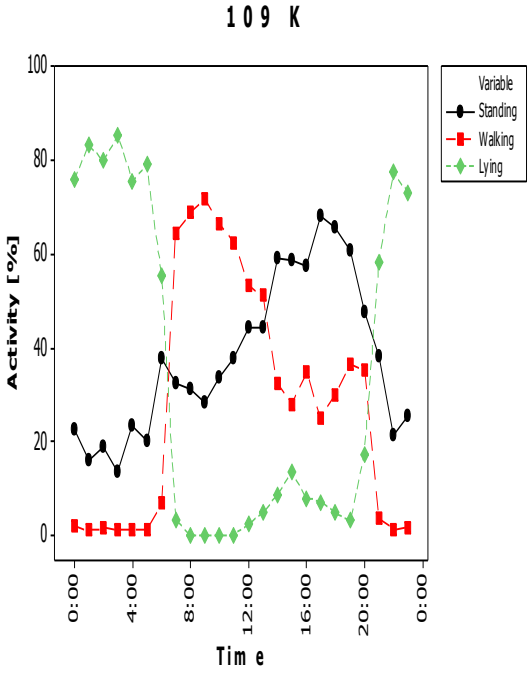

81 K
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00

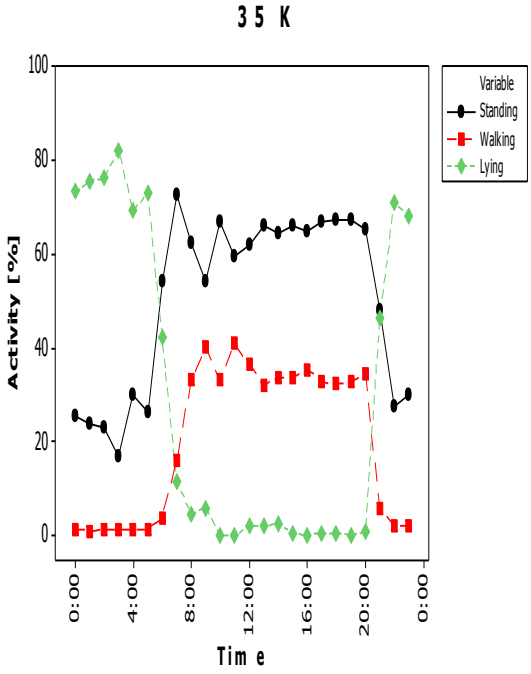

19 K
Activity [%]
Time
Variable
Standing
Walking
Lying
0:00
4:00
8:00
12:00
16:00
20:00
0:00

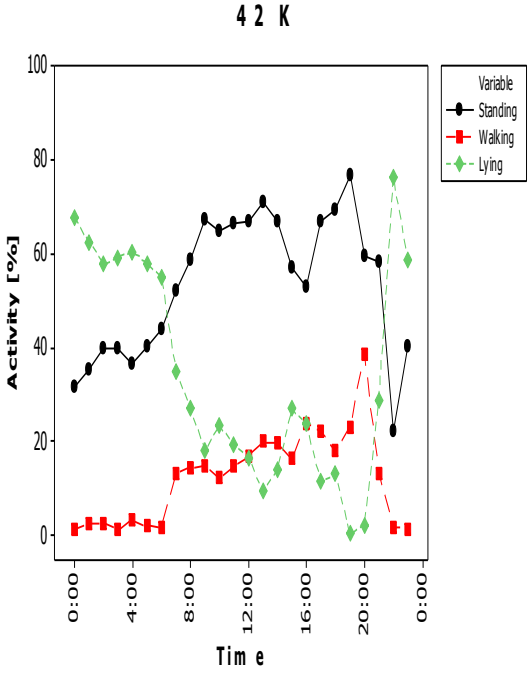

8 K
Activity [%]
Time
Variable
Standing
Walking
Lying
0:00
4:00
8:00
12:00
16:00
20:00
0:00

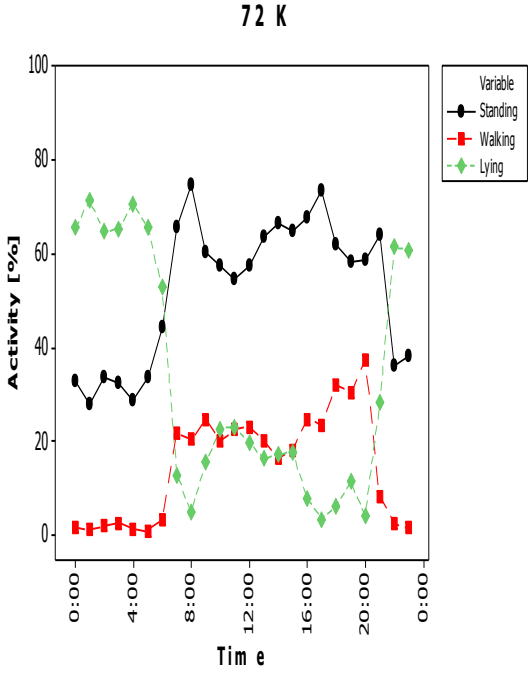

61 K
Activity [%]
Time
Variable
Standing
Walking
Lying
0:00
4:00
8:00
12:00
16:00
20:00
0:00


11 K
Activity [%]
Time
Variable
Standing
Walking
Lying
0:00
4:00
8:00
12:00
16:00
20:00
0:00

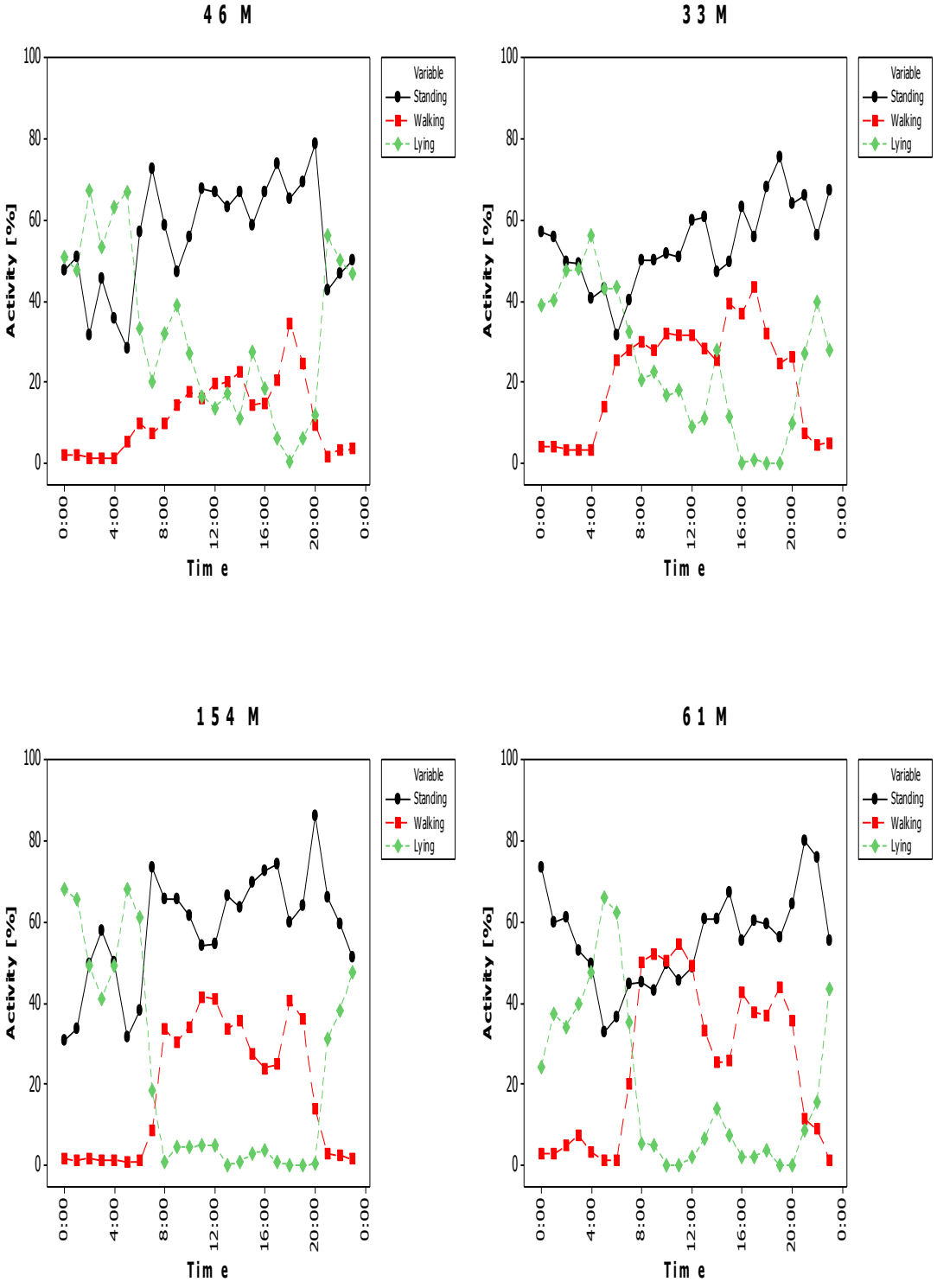

109 K
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00

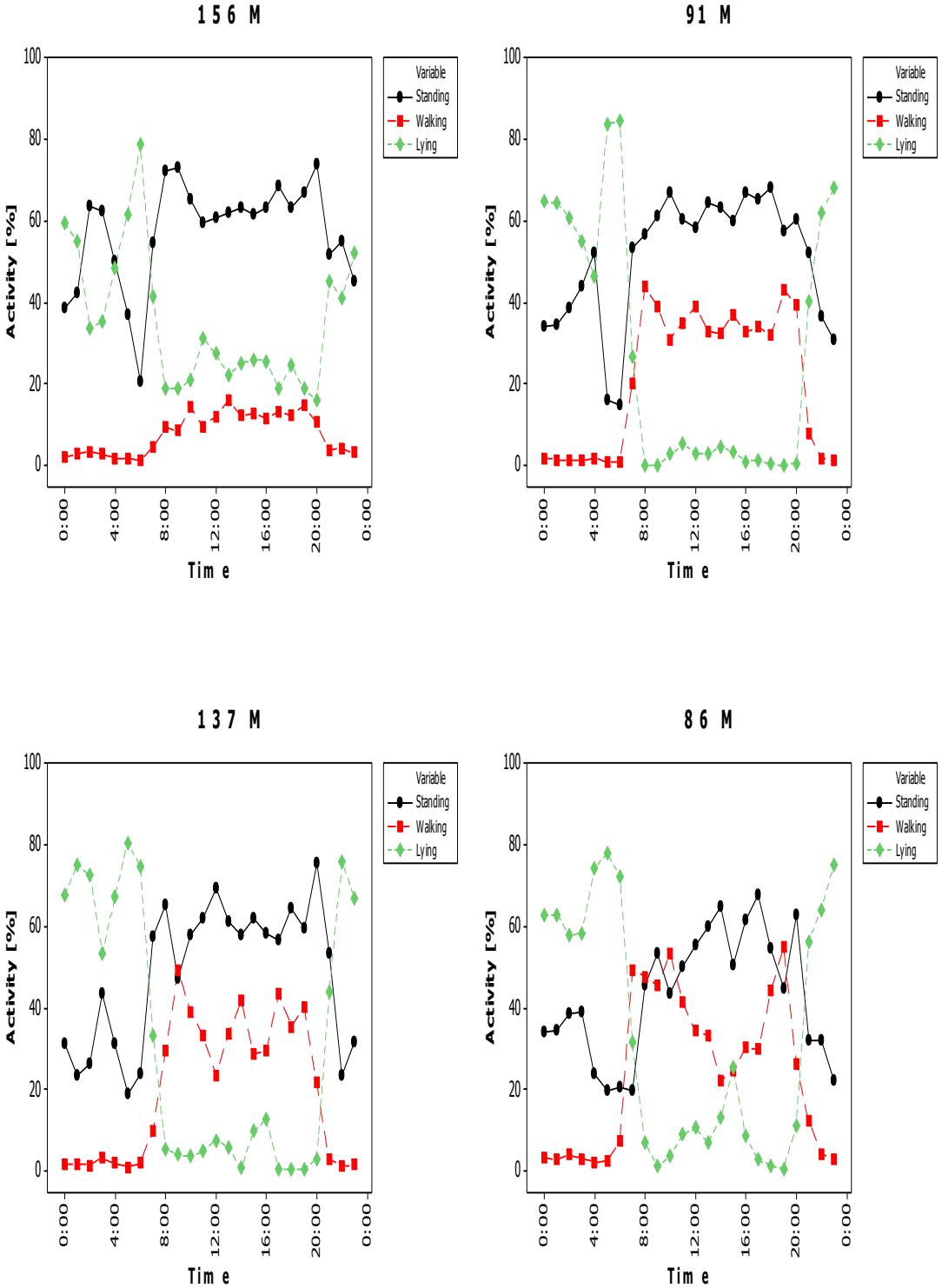

35 K
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00

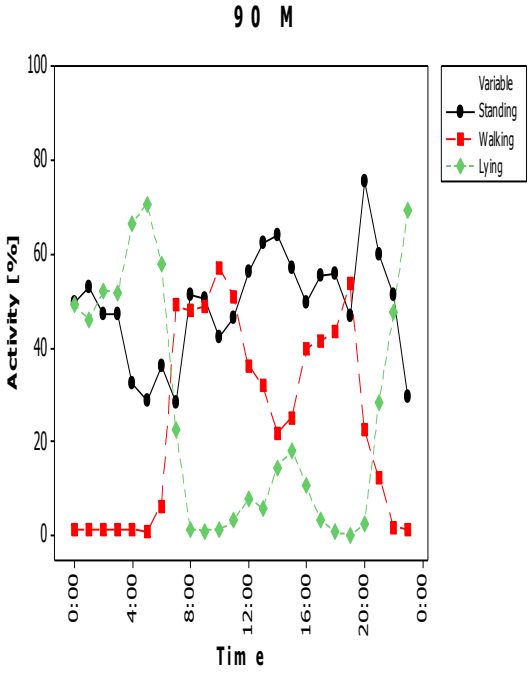

42 K
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00

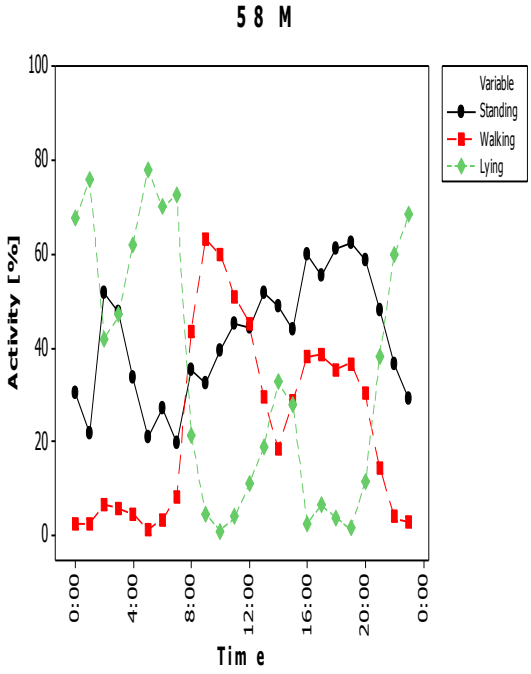

72 K
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00

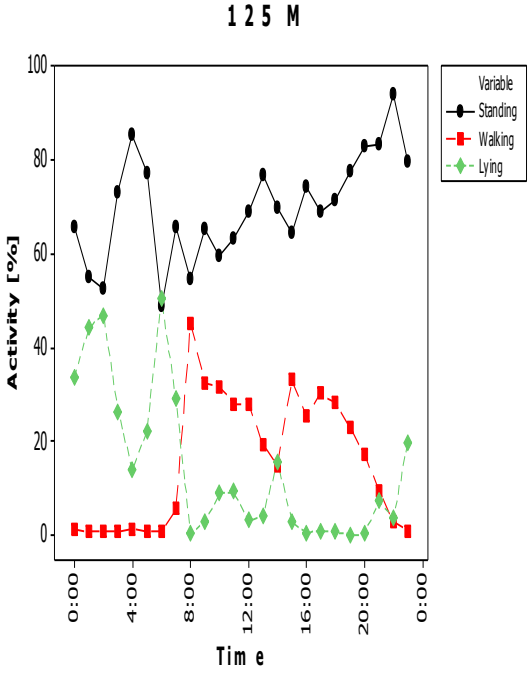
Appendix 7: Movement behaviour profiles of cattle pairs indicating percentage of time spent standing, walking or lying down in Makale during a two week period during the 2006/07 study. The data point for each behaviour variable at each hour is the mean behaviour of the animal during this period. Cattle on the left are in the low haemoglobin group.




156 M
91 M
137 M
86 M
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00


90 M
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00


58 M
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00


125 M
Activity [%]
Time
Variable
Standing
Walking
Lying
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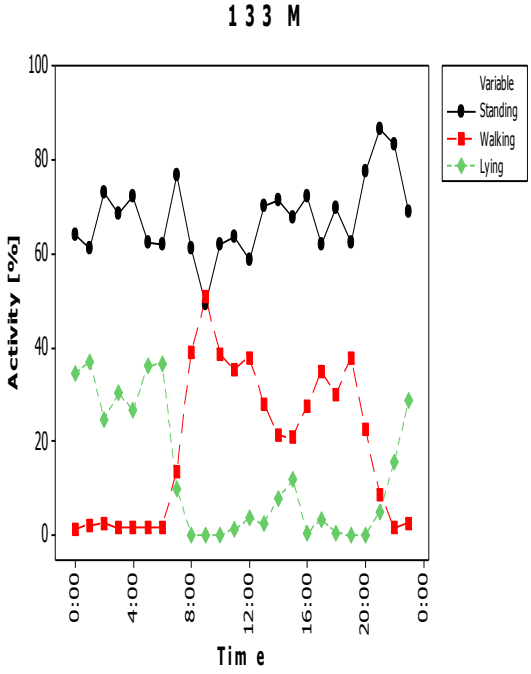
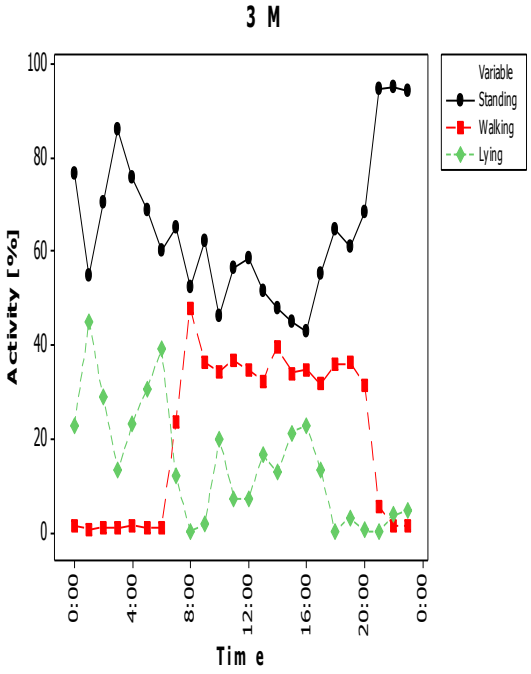
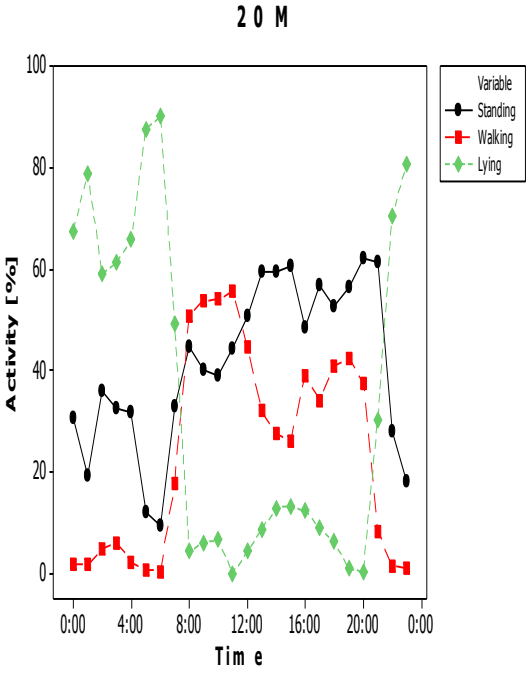
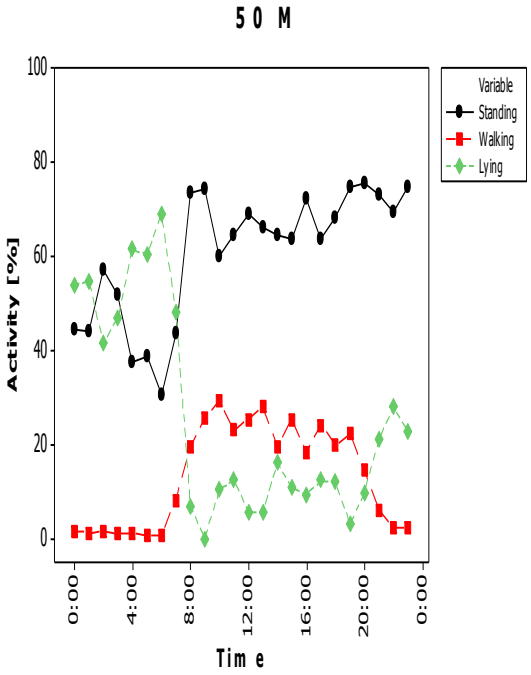
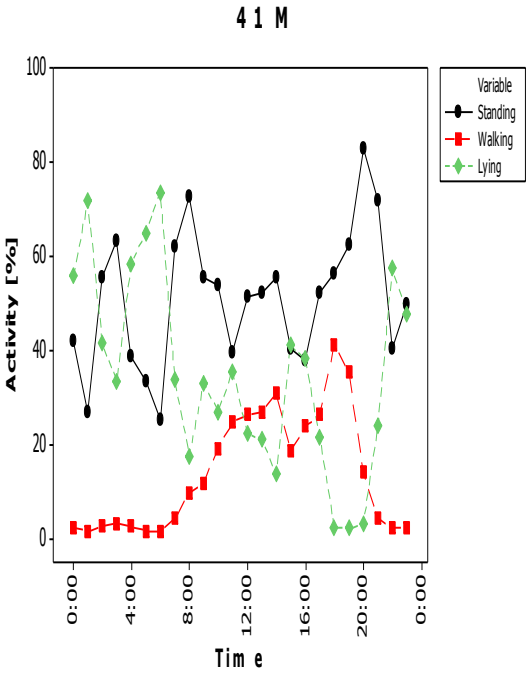
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00

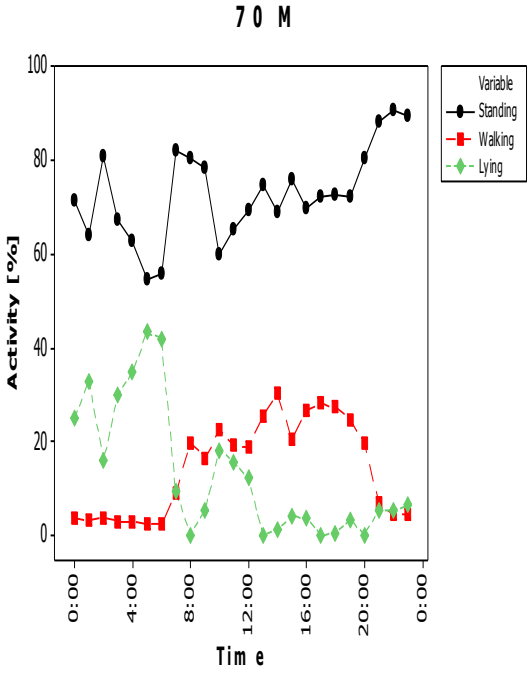

133 M
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00

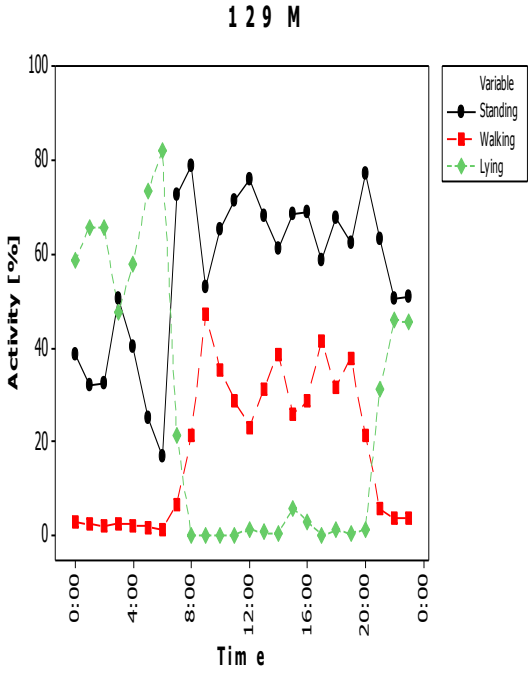

3 M
Activity [%]
Time
Variable
Standing
Walking
Lying
0:00
4:00
8:00
12:00
16:00
20:00
0:00

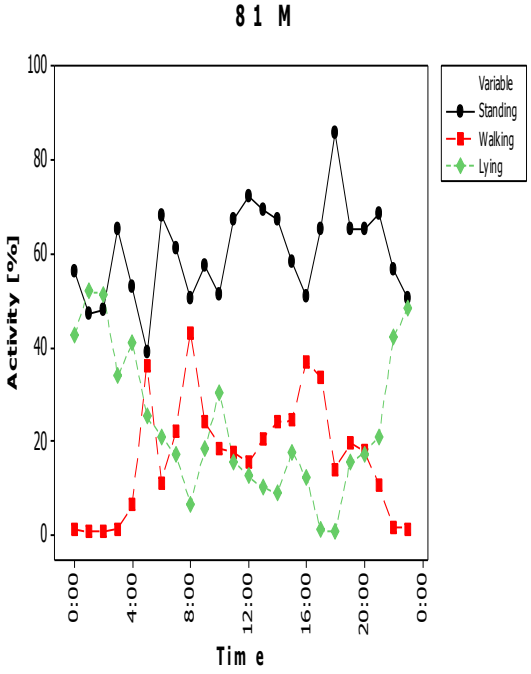

20 M
Activity [%]
Time
Variable
Standing
Walking
Lying
0:00
4:00
8:00
12:00
16:00
20:00
0:00

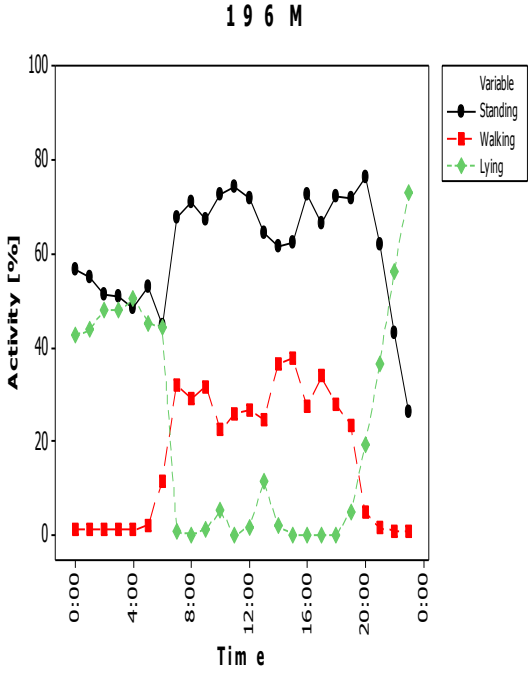

50 M
Activity [%]
Time
Variable
Standing
Walking
Lying
0:00
4:00
8:00
12:00
16:00
20:00
0:00


41 M
Activity [%]
Time
Variable
Standing
Walking
Lying
0:00
4:00
8:00
12:00
16:00
20:00
0:00

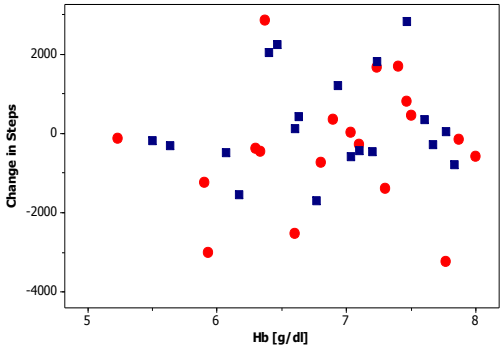

70 M
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00


129 M
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00

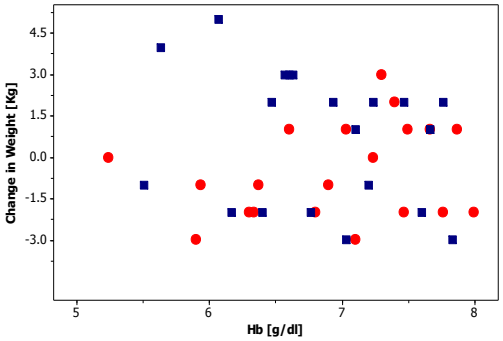

81 M
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00


196 M
Activity [%]
Time
Variable
Standing
Walking
Lying
0
20
40
60
80
100
0:00
4:00
8:00
12:00
16:00
20:00
0:00

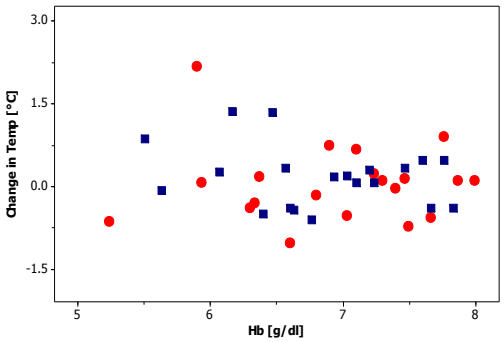
Appendix 8a: Difference of steps, haemoglobin, temperature and weight between week -1 and week 1 plotted against week -1 haemoglobin and steps in the Makale treatment study. Pearson correlation coefficients and p values for the Pearson's correlation are shown.



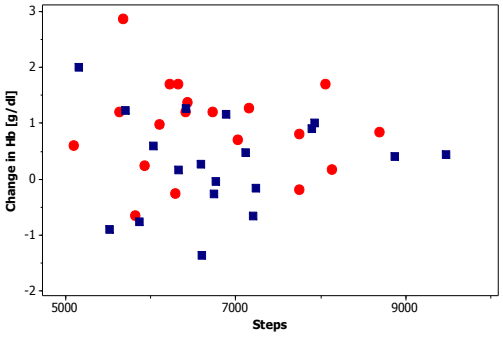
control(■) (pc = 0.11, p = 0.65), treated (●) (pc = 0.10, p = 0.68)



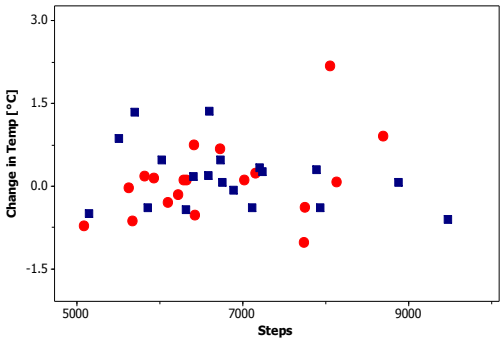
control (■) (pc = -0.25, p = 0.29), treated (●) (pc = 0.24, p = 0.30)



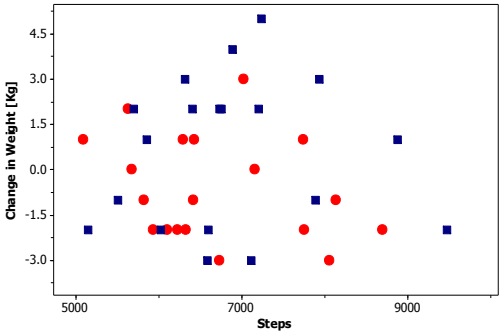
control (■) (pc = -0.28, p = 0.23), treated (●) (pc = -0.04, p = 0.88)



control (■) (pc = -0.09, p = 0.73), treated (●) (pc = -0.07, p = 0.78)



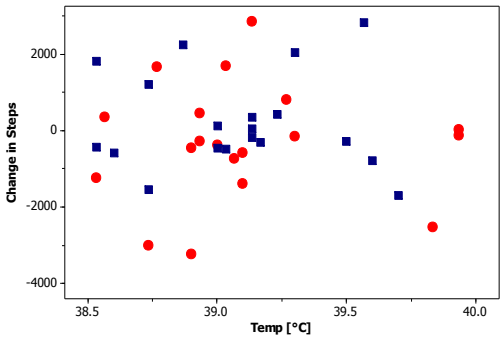
control (■) (pc = -0.35, p = 0.14), treated (●) (pc = 0.46, p = 0.05)



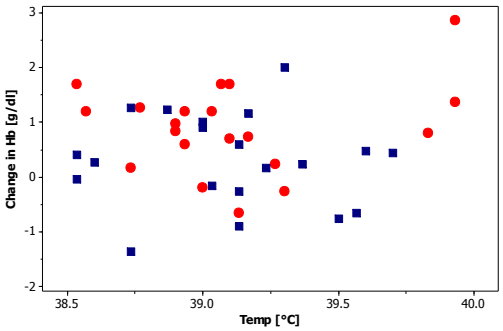
control (■) (pc = 0.05, p = 0.84), treated (●) (pc = -0.28, p = 0.25)

pc = Pearson correlation coefficient, p = p-value

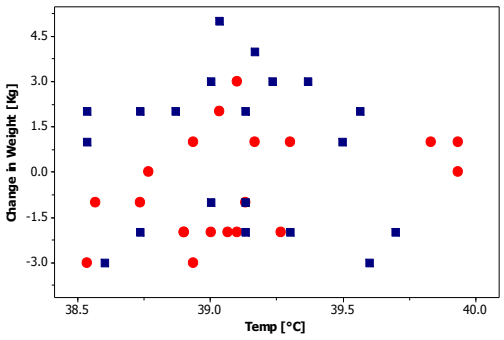
Appendix 8b: Difference of steps, haemoglobin, temperature and weight between week -1 and week 1 plotted against week -1 temperature and weight in the Makale treatment study. Pearson correlation coefficients and p values for the Pearson's correlation are shown.



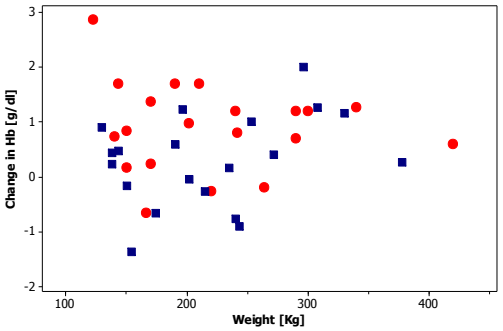
control (■) (pc = -0.04, p = 0.87), treated (●) (pc = 0.01, p = 0.97)



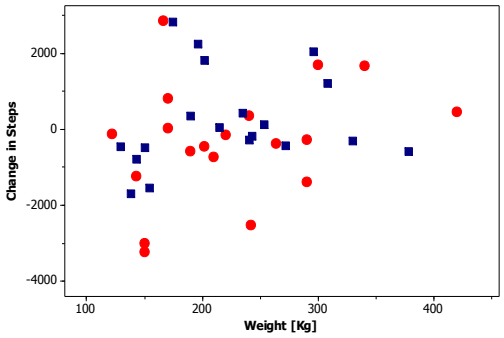
control (■) (pc = -0.13, p = 0.59), treated (●) (pc = 0.18, p = 0.48)



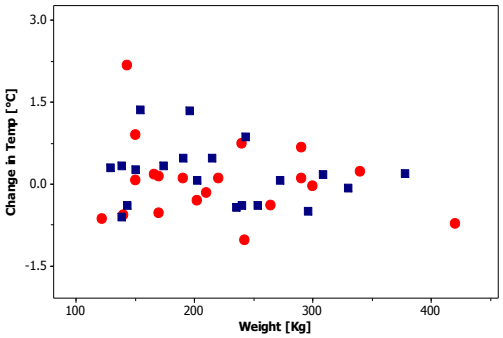
control (■) (pc = -0.07, p = 0.75), treated (●) (pc = , p = 0.07)



control (■) (pc = 0.27, p = 0.24), treated (●) (pc = , p = 0.59)



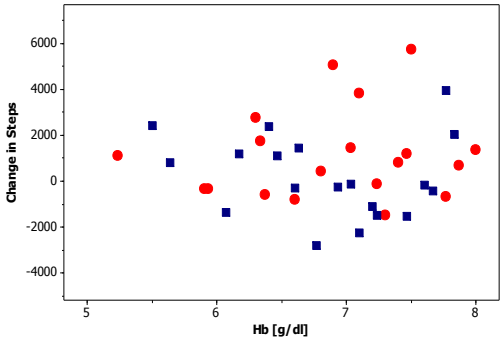
control (■) (pc = 0.16, p = 0.52), treated (●) (pc = 0.30, p = 0.21)



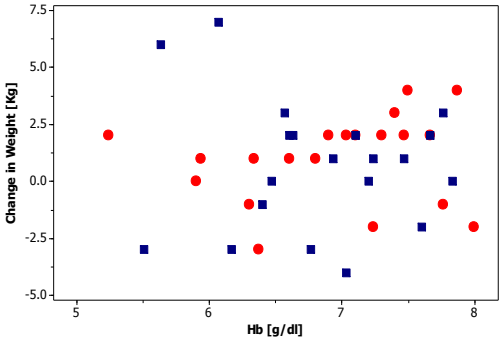
control (■) (pc = -0.09, p = 0.72), treated (●) (pc = -0.12, p = 0.65)

pc = Pearson correlation coefficient, p = p-value

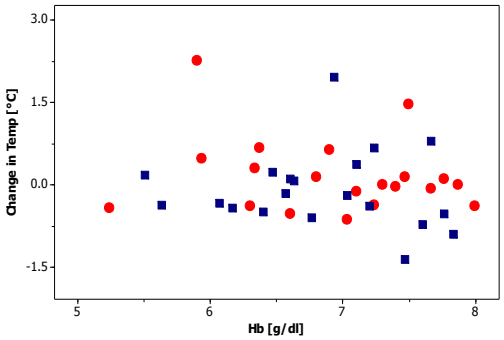
Appendix 8c: Difference of steps, haemoglobin, temperature and weight between week -1 and week 2 plotted against week -1 haemoglobin and steps in the Makale treatment study. Pearson correlation coefficients and p values for the Pearson's correlation are shown.



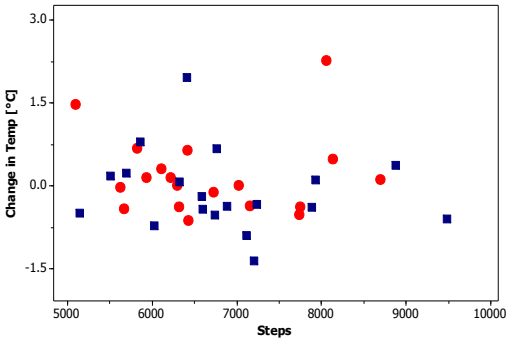
control (■) (pc = -0.13, p = 0.60), treated (●) (pc = 0.11, p = 0.63)



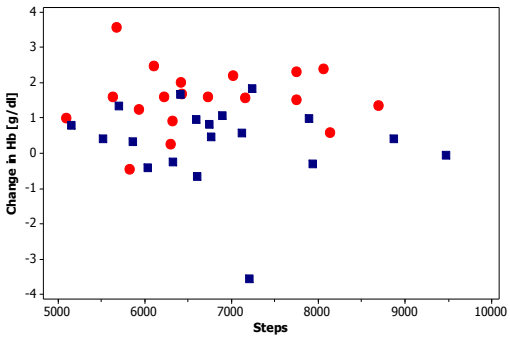
control (■) (pc = -0.1, p = 0.68), treated (●) (pc = 0.16, p = 0.51)



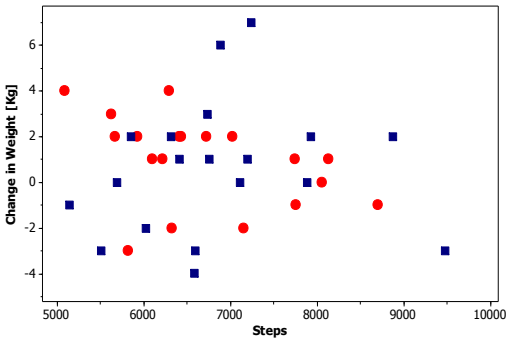
control (■) (pc = -0.15, p = 0.53), treated (●) (pc = -0.19, p = 0.43)



control (■) (pc = -0.16, p = 0.52), treated (●) (pc = -0.07, p = 0.79)



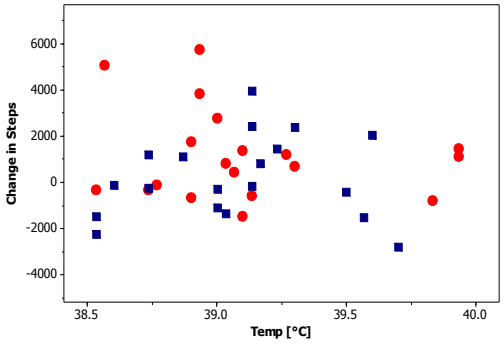
control (■) (pc = -0.12, p = 0.61), treated (●) (pc = -0.01, p = 0.96)



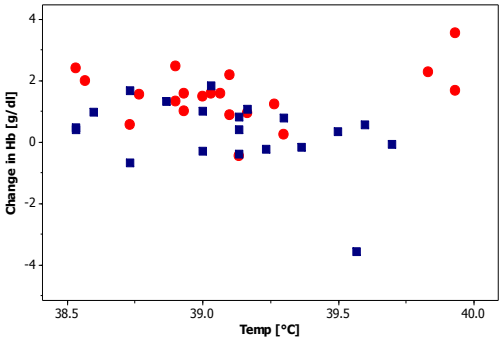
control (■) (pc = 0.11, p = 0.63), treated (●) (pc = -0.41, p = 0.08)

pc = Pearson correlation coefficient, p = p-value

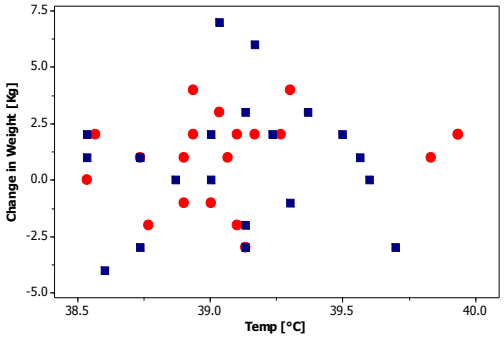
Appendix 8d: Difference of steps, haemoglobin, temperature and weight between week -1 and week 2 plotted against week -1 temperature and weight in the Makale treatment study. Pearson correlation coefficients and p values for the Pearson's correlation are shown.



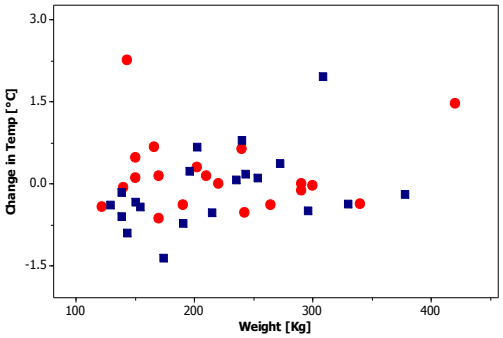
control (■) (pc = 0.10, p = 0.67), treated (●) (pc = -0.18, p = 0.45)



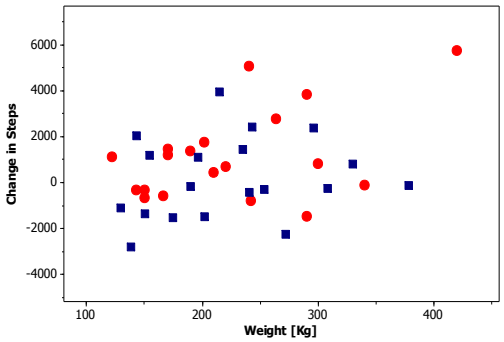
control (■) (pc = -0.29, p = 0.22), treated (●) (pc = 0.26, p = 0.27)



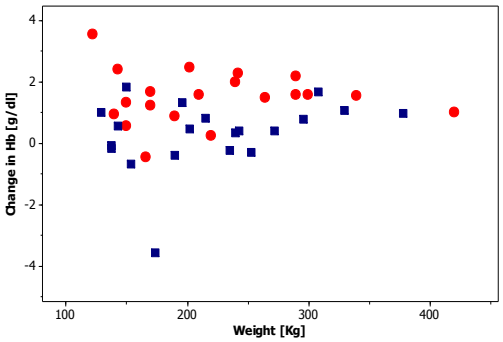
control (■) (pc = 0.06, p = 0.78), treated (●) (pc = 0.22, p = 0.36)



control (■) (pc = 0.32, p = 0.17), treated (●) (pc = 0.04, p = 0.88)



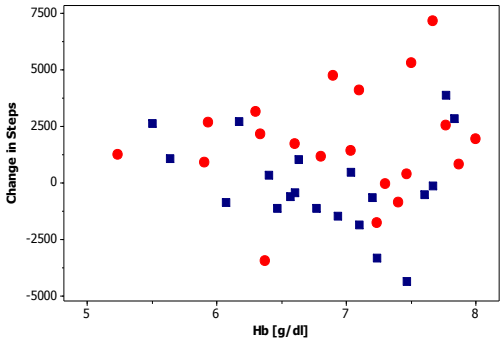
control (■) (pc = 0.18, p = 0.46), treated (●) (pc = 0.46, p < 0.05)



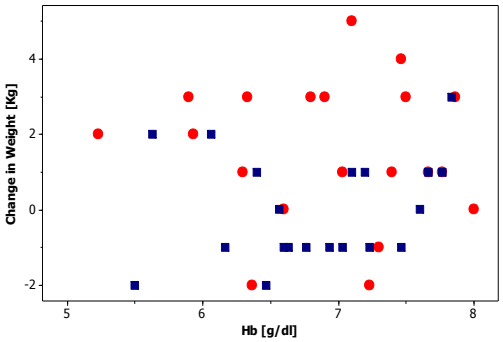
control (■) (pc = 0.26, p = 0.27), treated (●) (pc = 0.02, p = 0.95)

pc = Pearson correlation coefficient, p = p-value

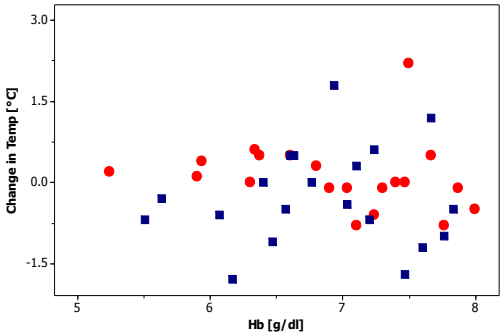
Appendix 8e: Difference of steps, haemoglobin, temperature and weight between week 1 and week 2 plotted against week -1 haemoglobin and steps in the Makale treatment study. Pearson correlation coefficients and p values for the Pearson's correlation are shown.



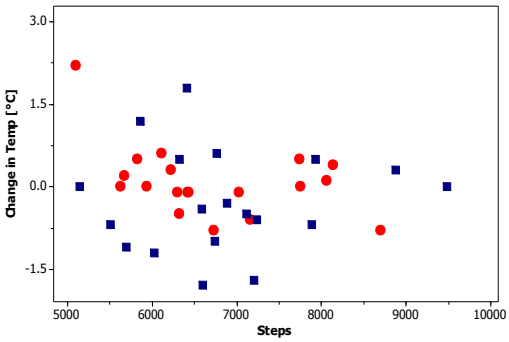
control (■) (pc = -0.17, p = 0.48), treated (●) (pc = 0.15, p = 0.52)



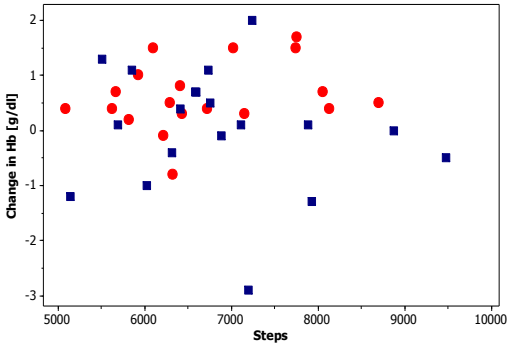
control (■) (pc = 0.23, p = 0.33), treated (●) (pc = -0.06, p = 0.80)



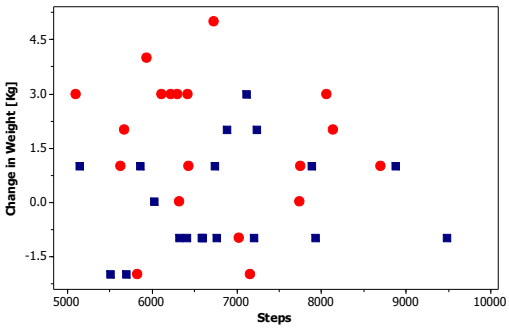
control (■) (pc = 0.03, p = 0.90), treated (●) (pc = -0.17, p = 0.46)



control (■) (pc = 0.007, p = 0.98, treated (●) (pc = -0.41, p = 0.08)



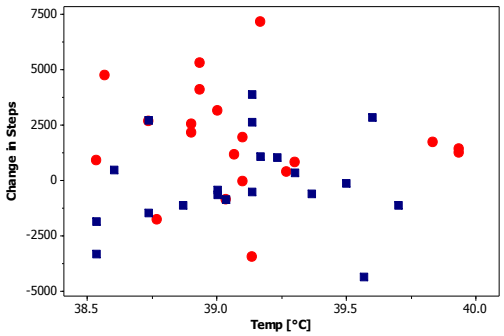
control (■) (pc = -0.20, p = 0.41), treated (●) (pc = 0.31, p = 0.20)



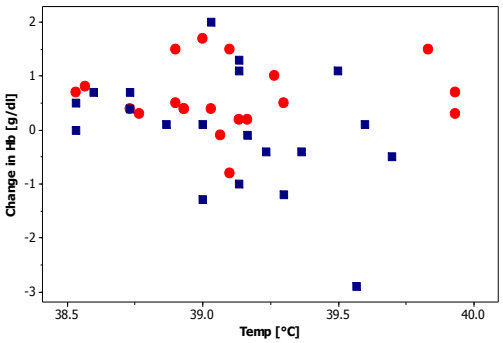
control (■) (pc = 0.15, p = 0.53), treated (●) (pc = -0.17, p = 0.50)

pc = Pearson correlation coefficient, p = p-value

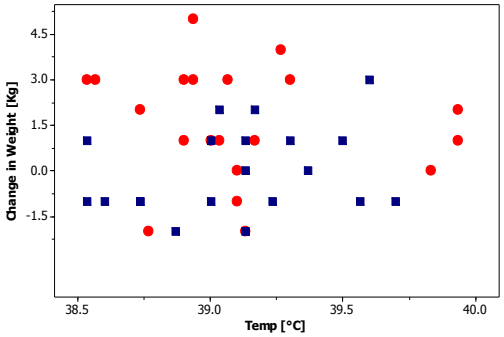
Appendix 8f: Difference of steps, haemoglobin, temperature and weight between week 1 and week 2 plotted against week -1 temperature and weight in the Makale treatment study. Pearson correlation coefficients and p-values for the Pearson's correlation are shown.



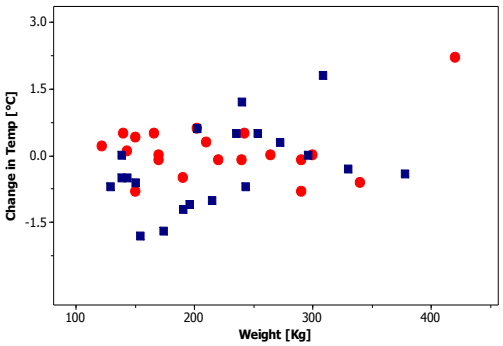
control (■) (pc = 0.1, p = 0.68), treated (●) (pc = -0.12, p = 0.61)



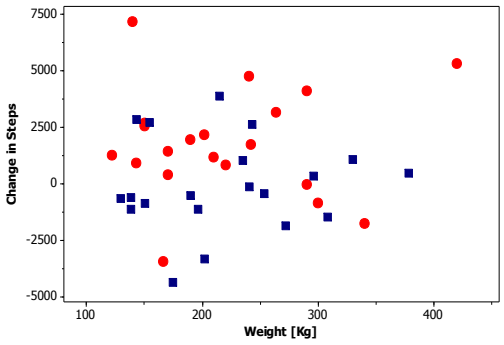
control (■) (pc = -0.46, p = 0.12), treated (●) (pc = 0.11, p = 0.65)



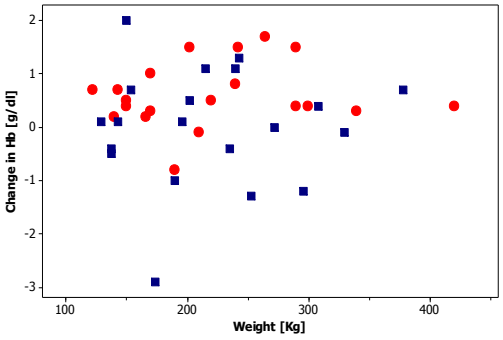
control (■) (pc = 0.26, p = 0.26), treated (●) (pc = -0.16, p = 0.51)



control (■) (pc = 0.55, p = 0.053), treated (●) (pc = 0.15, p = 0.52)



control (■) (pc = 0.07, p = 0.76), treated (●) (pc = 0.04, p = 0.86)



control (■) (pc = 0.08, p = 0.75), treated (●) (pc = -0.02, p = 0.94)

pc = Pearson correlation coefficient, p = p-value

Appendix 9: Cattle Management Questionnaire: Petauke District, Eastern Province Zambia 2008

Part A must be completed before any other part

Part A

Name of interviewer :(VO/VA)…….

Veterinary Camp…….……

Village…….……

GPS Coordinates…….……

Date……Time……..

Part B

Demographic

1. Name of respondent……...……
2. Gender of respondent (circle appropriate): **Male/Female**
3. Year of birth of respondent……..……
4. Relationship of respondent to the head household (**Self**)… ……..
5. How many people are in this household? Total……

   Children (upto 12 years?)……

   Children (13 -18yrs?)…….…….

   Adults (>18yrs)…….…….……..
6. Do the children go to school? (circle appropriate) **Yes/No**
7. If yes to the above, how many go to school?**.....**

   Children (upto 12 years?) that go to school……...

   Children (13 -18yrs?) that go to school ……
8. What times are they at school? **From**……..
   To…….……
9. Do members of your household go to church? (circle appropriate) **Yes/No**
   If yes, how many?**.....**

10. If yes to above, on which day(s) and times do you go to church:
    Day……..From…..……To…….……

11. What kind of farming system do you practice? (circle appropriate)…Mixed, livestock, crop, other……..……..

Livestock Inventory

1. How many cattle do you have (including calves):
   Total…….

2. Bulls……., Oxen…….., cows……..,
   calves……

3. Do you keep any other livestock? (circle appropriate) **Yes/No**

4. If yes, which ones
   (List)……..…….
   ……

Cattle management

1. Who herds your cattle.....……..…….

2. What time do your animals go out and come back from grazing (approximate)?
   Time they go out:…..…….Time they come back……

3. Are there differences to the way you manage your cattle during weekends and during week days? (circle appropriate) **Yes/No**

4. If Yes to the above, What are the differences
   ….....
   .....
   .....
   .....

5. Do you reward the people (person) herding your cattle? (circle appropriate) **Yes/No**

6. If yes, how do you reward them…….
   …….

7. Where do you keep your cattle at night?.....

8. Do you keep your cattle with other people's cattle at any time? **(Yes/No)**

9. If yes for the above, why?
   .....

.....
.....
.....

10. Who usually first notices that an animal is sick?.....

11. Who treats your sick animals? .....

12. Where does the medicine to treat your sick animals come from?
    .....
    ……

13. Do you use draft power? **(Yes/No)** ….If yes, for what purpose…….....
    ……

14. If yes, are the animals you use for draft power your own? **(Yes/No)**

15. If no, where do you get the animals from and do you pay for them?
    ……
    ……

16. Where do your cattle drink water from?
    A) In the dry season.....
    B) In the wet season.....

17. Where do your animals graze? (circle appropriate)
    A) Dry season: Dambos, plains, around village, Other……...
    B) Wet season: Dambos, plains, around village,Other……..

18. What are the cattle production constraints for you as a farmer?
    (Number them in the order they are given by respondent.)
    - Cattle diseases……..
    - Water……
    - Grazing land…….
    - Stock theft……
    - Wildlife……
    - Other 1:……..
    - Other 2:……..
    - Other 3:……..

19. Which are the two most important disease constraints in this area?

    Number 1 disease constraint ..……...

    Number 2 disease constraint……..